1996

Effects of transgenic Bacillus thuringiensis (Bt) corn on secondary lepidopteran pests and nontarget predators

Clinton Drake Pilcher
Iowa State University

Effects of transgenic *Bacillus thuringiensis (Bt)* corn on secondary lepidopteran pests and nontarget predators

by

Clinton Drake Pilcher

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Entomology
Major: Entomology

Approved:

Signatures have been redacted for privacy

iiversity
wa

1996

I dedicate this thesis to my parents, Stan and Marilyn, and my sister,
Jodi who have always been very supportive in helping
me accomplish my academic goals

TABLE OF CONTENTS

GENERAL INTRODUCTION 1

Thesis Organization 1

Literature Review 1

Development of transgenic crops 1

History of *Bacillus thuringiensis* 3

Development of transgenic *Bt* corn 8

Concerns regarding transgenic *Bt* corn 10

Objectives 13

1. PREIMAGINAL DEVELOPMENT AND FIELD ABUNDANCE OF INSECT PREDATORS ON TRANSGENIC *BACILLUS THURINGIENSIS* CORN 14

Abstract 14

Introduction 15

Materials and Methods 17

Laboratory Studies 17

Field Study 23

Results 24

Coleomegilla maculata 24

Orius insidiosus 25

Chrysoperla carnea 25

Predator Survey 26

Discussion 28

Acknowledgements 35

References Cited 36

2. FIELD AND LABORATORY EVALUATIONS OF TRANSGENIC *BACILLUS THURINGIENSIS (BT)* CORN ON SECONDARY LEPIDOPTERAN PESTS 54

Abstract 54

Introduction 55

Materials and Methods 58

Field Studies 58

Laboratory Studies 59

Agrotis ipsilon 61

Papaipema nebris 63

Pseudaletia unipuncta 64

Helicoverpa zea 65

Results 66

Agrotis ipsilon 66

Papaipema nebris 67

Pseudaletia unipuncta 67

Helicoverpa zea 69

Discussion 70

Acknowledgements 76

References Cited 76

GENERAL SUMMARY 96

REFERENCES CITED 99

ACKNOWLEDGMENTS 105

GENERAL INTRODUCTION

Thesis Organization

This thesis is organized in the following manner: a general introduction including literature review and thesis objectives, the two papers prepared for publication in scientific journals, a general summary, references cited, and acknowledgements. The first paper addresses the first and second objectives, while the second paper addresses the third and fourth objectives. The references cited section at the end of the thesis contains all references cited in the general introduction and summary. The first paper was prepared for submission to Environmental Entomology and the second paper was prepared for submission to the Journal of Economical Entomology. Both papers were prepared for submission based on guidelines of the Entomological Society of America. All references listed follow the format of the Entomological Society of America.

Literature Review

Development of transgenic crops. One goal of biotechnology in agriculture has been the development of pest-resistant plants. Genetically engineering plant cells to express genes with specific qualities is a revolutionary approach for managing crop pests (diseases, weeds, and insects). Since 1983, more than 20 different plant species have been successfully transformed using different biochemical systems (Peferoen & Mellaert 1991). Transformation is the

process of taking a foreign gene sequence and placing it into the nuclear genome (DNA) of another organism, thus the term transgenic.

Transgenic crops have been developed using several different molecular methods. The most commonly used procedure to produce transgenic plants is the *Agrobacterium tumerfaciens* system (Gasser & Fraley 1989, Steinbiss & Davidson 1989, Peferoen & Mellaert 1991). *Agrobacterium tumerfaciens* is a soil bacterium that infects many dicotyledonous and gymnospermous plants if they have been wounded (Steinbiss & Davidson 1989, Fosket 1994). This system involves the transfer of a functional gene (insect resistance, herbicide tolerance, etc.) into host DNA by means of a vector system (Grierson & Covey 1988, Fosket 1994). This system works very well, but is primarily limited to dicotyledonous plants.

Successful gene transformation has also taken place in monocotyledonous plants (rice, wheat, and corn) by means of direct DNA transfer systems (Fromm et al. 1990, Fujimoto et al. 1993, Vasil et al. 1993, 1994). One simple technique consists of protoplast incubation with plasmid DNA in the presence of polyethylene glycol (PEG) (Steinbiss & Davidson 1989, Vasil 1994). Another technique commonly used in conjunction with PEG is electroporation. Electroporation uses short, high energy electrical impulses to increase the permeability of the plasma membrane allowing foreign DNA uptake (Murphy & Thompson 1988). However, it is very difficult to regenerate plants from protoplasts, which is what PEG and electroporation gene transfer systems use.

Because of this, a different method of producing transgenic crops has been developed using a particle bombardment system where tungsten particles (microprojectiles) are loaded with the gene of choice and shot into plant cells using a particle gun (Gasser & Fraley 1989, Steinbiss & Davidson 1989, Fromm et al. 1990, Fujimoto et al. 1993, Koziel et al. 1993, Vasil et al. 1993, 1994). The particles are able to penetrate the cell wall and plasma membrane allowing transfer of the selected gene. This technique, in addition to those described, have been very useful tools in evaluating and producing transgenic plants which will benefit growers in the following areas: crop quality, resistance against diseases and insects, and protection from herbicides used to control weeds.

These techniques have been used to develop many transgenic field and vegetable crops: wheat, corn, rice, tobacco, cotton, soybean, cabbage, tomatoes, and potatoes (Fromm et al. 1990, Beegle & Yamamoto 1992, Warren et al. 1992, Benedict et al. 1993, Fujimoto et al. 1993, Murry et al. 1993, Perlak et al. 1993, Vasil et al. 1993). One gene widely used to transform plants is the gene encoding the ∂ - endotoxin produced by *Bacillus thuringiensis* Berliner (*Bt*). Corn, potato, cabbage, tomato, soybean, cotton, tobacco, and rice have been modified to functionally express the ∂ - endotoxin derived from *Bt* (Kumar & Sharma 1994).

History of *Bacillus thuringiensis*. The initial discovery of *Bt* occurred in 1901 when Japanese bacteriologist S. Ishiwata isolated the bacillus from diseased *Bombyx mori* L. larvae (Beegle & Yamamoto 1992, Knowles 1994). He described

the pathology it causes in silkworm larvae. Ishiwata noted that there had to be some type of toxin involved in the pathogenicity (Beegle & Yamamoto 1992). Ten years later, Ernst Berliner isolated a similar organism and named it *Bacillus thuringiensis* (Beegle & Yamamoto 1992). He was the first to describe the bacterial species as being a gram-positive, rod-shaped, spore-forming bacterium that was present in soil (Beegle & Yamamoto 1992).

Due to a serious problem with the European corn borer, *Ostrinia nubilalis,* (Hübner), in North America, the United States funded the International Group for Corn Borer Investigation in the late 1920s (Beegle & Yamamoto 1992). Depressed economic conditions in the 1930s in North America resulted in an end to the funding of the project using *Bt* as a control agent for *O. nubilalis.* In the early 1950s, Edward Steinhaus published several articles that stimulated interest in the use and commercial development of *Bt* as a microbial control agent of lepidopteran pests (Beegle & Yamamoto 1992). However, formulations of *Bt* products could not compete economically with cheaper broad spectrum insecticides. The first commercial preparation of a *Bt* product finally occurred in 1970 when Abbott Laboratories entered the market with Dipel® (Beegle & Yamamoto 1992).

The discovery and development of the subspecies *kurstaki* and specifically isolate HD-1 in 1970, named by Dulmage, instigated the search for new strains of the bacterium (Beegle & Yamamoto 1992). Several subspecies of *Bacillus thuringiensis* have been isolated; a few include: *kurstaki, aizawai, sotto,*

entomocidus, berliner, thuringiensis, tenebrionis, israelensis, and *morrisoni* (Höfte & Whiteley 1989). When *Bt* sporulates, it produces a parasporal crystal inclusion body. This crystal protein is produced by genes that are referred to as *cry* genes. The proteins encoded by these genes are classified according to structural similarities and insecticidal specificity (Höfte & Whiteley 1989). The four main subclasses are as follows: Cry1 proteins are lepidopteran-specific and have a molecular weight of approximately 130 kDa, Cry2 proteins are dipteran/lepidopteran-specific and have a molecular weight of 70 kDa, Cry3 proteins are coleopteran-specific and have a molecular weight of 70 kDa, and Cry4 proteins are dipteran-specific including 70- and 130 kDa size proteins (Höfte & Whiteley 1989, Gill et al. 1992, Yamamoto & Powell 1993, Knowles 1994, Crickmore 1995).

Activity of *Bt* in the gut of the host organism is dependent on two characteristics: 1) differences in the larval gut affecting the solubilization and/or processing efficiency of the protoxin and 2) the presence of specific toxin-binding sites (receptors) in the gut of different insects (Höfte & Whiteley 1989). The insect ingests the protein, in the form of a protoxin, into an alkaline gut containing protease enzymes which cleave the protoxin into a structural and active fragment (Höfte & Whiteley 1989, Gill et al. 1992, Knowles 1994, Kumar & Sharma 1994). The active fragment is toxic and binds to specific receptors in the target gut epithelial lining which causes the cells to swell and eventually burst (Höfte & Whiteley 1989, Kumar & Sharma 1994). The exact mechanism by which

the toxic fragment binds causing the cell to swell and burst is currently unknown (Knowles 1994). The cells bursting causes a sloughing of the gut epithelium which causes the insect to stop feeding and eventually die if a lethal dose has been ingested.

There are several advantages in using *Bt* to control crop pests including: 1) narrow host range, 2) little to no residual, 3) safe for growers to handle, and 4) harmless to vertebrates. The disadvantages of *Bt* include: 1) no immediate observed effect (activity of *Bt* appears to be slow to growers compared to synthetic insecticides), 2) several applications of *Bt* product may be needed for higher efficacy due to UV breakdown of the protein, 3) timing of application of *Bt* microbial insecticides is critical, 4) exact placement of the product to where the insect is feeding is required, and 5) specificity may be considered a disadvantage because growers often need to control more than one pest.

The advantages of *Bt* fit well into a basic goal of integrated pest management (IPM) which is to keep pest populations below the economic injury level while preserving naturally occurring beneficial organisms (Pedigo 1995). Hollander (1991) states that the opportunity to substitute safer, more selective, and biodegradable pest control agents provides important environmental benefits for society. These benefits are becoming increasingly important factors when growers decide what methods to use in controlling insect pests.

There are many commercial *Bt* products that are being utilized for the management of agricultural and forest pests. *B.t. israelensis* is is used as a

mosquito larvicide. *B.t. tenebrionis* is active against leaf feeding coleopteran larvae including Colorado potato beetle, *Leptinotarsa decemlineata*. However, over two-thirds of *Bt* based products utilize strains from the subspecies *kurstaki* which are active against over 55 lepidopteran species, including *O. nubilalis* (Adang 1991, Beegle & Yamamoto 1992).

Ostrinia nubilalis is susceptible to a number of strains and subspecies of *Bt* (Koziel et al. 1993). Commercial products which show activity against *O. nubilalis* include Dipel®, Javelin®, Larvin®, M-Peril®, and MVP® (ESA 1994). In general, these products have not been as effective as synthetic insecticides against *O. nubilalis* because of placement of the *Bt*, rapid breakdown of *Bt*, or the timing of the application. Commercial *Bt* microbial sprays are effective against *O. nubilalis*, but improved methods are needed given the severity of economic damage caused by this pest.

Yield losses in corn, *Zea mays* L., due to *O. nubilalis* damage in the Midwest has ranged from a low of 19.3 million bushels in 1974 to 138.6 million bushels in 1971 (Lynch 1980). Yield losses up to 6% per borer per plant can result from feeding at different stages during the growth of the plant (Bode & Calvin 1990). Chemical insecticides are commonly used throughout different regions in the Corn Belt to control corn borers, however, as observed with *Bt* products, difficulty in timing applications may result in the need for multiple treatments because of the duration of ovipositional activity of the female (Showers et al. 1992). *Ostrinia nubilalis* is most vulnerable to insecticidal control (including *Bt*

sprays) from hatch through third instar (Showers et al. 1992). There are other management tools which can be used including plant resistance and cultural control, but these methods are not always successful and their results are affected by numerous factors. In addition, biological control is another controlling factor of corn borer populations, but monitoring and determining the economic impact is difficult. If larvae begin to feed below the leaf sheath and bore into the stalk, control becomes impossible. However, biotechnology has made possible a new method to control *O. nubilalis* more effectively utilizing *Bt* by killing larvae before they enter the stalk.

Development of transgenic *Bt* corn. In 1993, Koziel et al. published the first report on the development of transgenic corn expressing an insecticidal crystal protein derived from *Bt* that had been developed. The *cry1Ab* gene produced from the *Bt* subspecies *kurstaki* HD-1 strain was incorporated into corn plants providing highly effective protection against *O. nubilalis*. Microprojectile bombardment of immature corn embryos allowed the insertion of *cry1Ab* genes into a proprietary inbred line, CG00526. Transcription of the *cry1Ab* gene was obtained by using either the cauliflower mosaic virus (CaMV) 35S promoter or a combination of the corn phosphoenolpyruvate carboxylase (PEPC) promoter and a pollen specific promoter (Koziel et al. 1993). Embryos from both transformation events were germinated and grown in peat pots. When sufficient leaf material was available, the following analyses were performed to verify protein presence and function: ß- glucuronidase (GUS) histochemical

assay, PCR analysis for transgenes, growth in the presence of PPT (phosphoinothricin, a selectable identifying marker), ELISA (enzyme linked immunosorbent assays) for Cry1Ab protein, and insect bioassays with *O. nubilalis* larvae (Koziel et al. 1993). These laboratory tests verified activity of the *Bt* protein within the corn leaf tissue.

Field efficacy tests were conducted in addition to laboratory analyses. Corn plants were infested with 300 first instars per week for eight weeks. The length of tunnels made by the larvae were significantly longer in isogenic (non*Bt*) plants than in transgenic *Bt* plants, and mortality of *O. nubilalis* ranged from 80-100 percent in the latter (Koziel et al. 1993). These results show the high efficacy against *O. nubilalis*, but it is not known if the protein expression will affect other insect species. The type of gene promoter, number of copies, and points of insertion are all important in determining the concentration and activity of a gene within various organs of a corn plant. The promoters and the level of expression (concentration of Cry1Ab/mg soluble protein) will determine what parts of a plant are protected and the effectiveness of control against *O. nubilalis*.

Transgenic *Bt* corn has several advantages and disadvantages compared to the use of *Bt* as a foliar microbial insecticide. Besides the initial advantages associated with *Bt* products (narrow host range, no residual, safety to growers, and harmlessness to vertebrates), transgenic *Bt* corn adds additional benefits including the following: plants provide protection throughout the growing season and in all parts of the plant, growers should be able to reduce scouting

time, and there are no application or additional labor costs involved. Transgenic *Bt* corn removes several disadvantages associated with *Bt* microbial sprays, but adds several potential others: insect resistance problems, growers ignoring pests in their fields, and unknown effects on nontarget insects.

Concerns regarding transgenic *Bt* corn. Transgenic *Bt* corn has been approved for registration by the Environmental Protection Agency (EPA), and will be released into agricultural fields in 1996 (Dean Christensen, Ciba Seeds, personal communication). Concerns arise as to whether enough short- and long-term research to determine the ecological impact of *Bt* toxins on the ecosystem has been completed (Raybould & Gray 1994).

Risk assessment is one part of the cost-benefit analysis that is used to determine whether or not to release a novel material (transgenic plants) into the environment (Jepson et al. 1994). Hollander (1991) proposed two possible environmental consequences of reliance on transgenic products: those which are agent-specific, resulting directly from the introduction of specific agents into the environment and those which cumulatively build up over time. Research has shown that nontarget lepidopteran species can be ecologically affected in large-scale pest control programs using *Bt* microbial sprays (Miller 1990). Should known facts about traditional *Bt* microbial sprays be applied to transgenic plants? In addition, will the effects not be compounded by placing *Bt* into a plant? No data currently exist that provides these answers.

The first stage in the risk assessment analysis of transgenic *Bt* corn is to determine the community of nontarget organisms that will most likely be effected. The *Bt* corn to be planted in 1996 expresses higher levels of *Bt* protein in the leaf and pollen than are normally observed by *Bt* spray applications (Koziel et al. 1993). Many predatory species that feed on *O. nubilalis* eggs and young larvae also feed on corn pollen (Smith 1965, Hodek 1973, Isenhour & Yeargan 1981, Andow 1990, Coll & Bottrell 1991). Three pollen-feeding predators that are common in corn fields are the twelve-spotted lady beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and the minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Sparks et al. 1966, Gordon 1985, Jarvis & Guthrie 1987).

Relatively few studies have assessed the effects transgenic organisms or microbial *Bt* sprays on nontarget natural enemies. Johnson and Gould (1992) studied parasitism of *Heliothis virescens* (F.) on transgenic tobacco and reported that *Bt* protein expressed at low levels in transgenic tobacco appears to be compatible with parasitoids for suppression of *H. virescens*. Two predators, *C. carnea* and *O. insidiosus* were not adversely affected by feeding on larvae of *H. virescens* infected with a recombinant nuclear polyhedrosis virus (Heinz et al. 1994). Larvae and adult *C. carnea* were not effected by being sprayed with two different *Bt* spray products (Melin & Cozzi 1990). Beetle populations were monitored following use of *Bt* microbial sprays and bait formulations on tobacco

where populations of two species, *Hippodamia convergens* Guerin-Meneville and *C. maculata* were not affected by the treatments (Melin & Cozzi 1990). Also, Sims (1995) showed no deleterious effects of *Bt* Cry1Ac protein expressed in transgenic cotton on several natural enemies including *H. convergens, C. carnea,* and *Nasonia vitripennis* (Walker). However, contrary to these studies, in a laboratory study, M-One, a *Bt* microbial spray used to control Colorado potato beetle, *Leptinotarsa decemlineata,* decreased the predation rate of *C. maculata* (Giroux et al. 1994). This study shows that predators can be affected by *Bt* and that there may be good reason to believe that high levels of *Bt* protein expressed in corn pollen may affect predators' feeding efficiencies and/or behavior in the field.

In addition to natural enemies, several secondary lepidopteran pests that may feed on transgenic *Bt* corn causing economic damage include: black cutworm (*Agrotis ipsilon* (Hufnagel)), stalk borer (*Papaipema nebris* (Geunée)), armyworm (*Pseudaletia unipuncta* (Haworth)), and corn earworm (*Helicoverpa zea* (Boddie)). Many studies report the susceptibility of these insects to different strains of *Bt* protein under laboratory conditions (Burges 1981, Jaquet et al. 1987, Höfte et al. 1988, MacIntosh et al. 1990, Stone & Sims 1993, Ebora et al. 1994), and in field conditions (Miller 1990, Ali & Young 1993, Bartels & Hutchison 1995, Johnson et al. 1995). However, only one study has examined the effects of transgenic plants (potato) on secondary lepidopteran insects (Ebora et al. 1994). They observed a significant impact on the primary pest, *Phthorimea operculella*

(Zeller) and secondary pest, *O. nubilalis,* in potatoes. Could transgenic *Bt* corn significantly effect secondary lepidopteran pests of corn?

Objectives

This research examines the effects of transgenic *Bt* corn on secondary lepidopteran pests and insect predators. The specific objectives are to:

1) determine effects of *Bt* corn pollen on the preimaginal development and survival of *C. maculata, C. carnea,* and *O. insidiosus.*

2) compare the temporal occurrence and abundance of insect predators on transgenic *Bt* corn and non*Bt* corn plants.

3) determine the susceptibility of *A. ipsilon, P. nebris, P. unipuncta,* and *H. zea* to transgenic *Bt* corn in the laboratory.

4) compare the field efficacy of transgenic *Bt* corn and non *Bt* corn against these corn pests.

PREIMAGINAL DEVELOPMENT AND FIELD ABUNDANCE OF INSECT PREDATORS ON TRANSGENIC *BACILLUS THURINGIENSIS* CORN

A paper to be submitted to *Environmental Entomology*

C. D. Pilcher, J. J. Obrycki, M. E. Rice & L. C. Lewis

Abstract

Laboratory studies were conducted to determine the effects of feeding corn pollen expressing a Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki* on three predatory species: *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Heteroptera: Anthocoridae), and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). No acute detrimental effects of the Cry1Ab protein on preimaginal development and survival were observed among these predators reared on *Bt* corn pollen. The following survival percentages were observed: *C. maculata*, 89 ± 2.2% (*Bt* corn pollen), 69 ± 5.9% (non *Bt* corn pollen), *O. insidiosus*, 63 ± 12% (*Bt* corn pollen), 44 ± 10.2% (non *Bt* corn pollen), *C. carnea*, 49 ± 3.5% (*Bt* and non *Bt* corn pollen). Additional studies are needed to test for chronic and reproductive effects over several generations before concluding that transgenic *Bt* corn pollen has no effect on insect predators. From two years of field evaluations, no differences were observed in the abundance of *Ostrinia nubilalis*, European corn borer predators (coccinellids, chrysopids, anthocorids) on *Bt* or non *Bt* corn. Predator numbers

observed during sampling before, during, and after pollen shed suggest no detrimental effects of *Bt* corn on natural enemy behavior.

Introduction

The European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), is an introduced pest of corn (*Zea mays* L.) that damages the plants' physiological processes and reduces grain yield. Historically, control has focused on importation of natural enemies, multiple timed insecticide applications, or use of corn hybrids expressing moderate resistance to *O. nubilalis* larvae (Clausen 1978, Barry & Darrah 1991, Showers et al. 1992). Several studies have determined that naturally occurring predators, parasitoids, and pathogens help regulate *O. nubilalis* populations (Sparks et al. 1966, Jarvis & Guthrie 1987, Coll & Bottrell 1991, Lewis & Bing 1991). Despite these managment approaches and biotic mortality, *O. nubilalis* remains a persistent pest of corn in the Midwest, causing losses between $15 and $70 per acre of corn (Rice 1994).

Three relatively abundant predators that feed on *O. nubilalis* eggs and young larvae in the central United States are the twelve spotted lady beetle, *Coleomegilla maculata* (DeGeer), the green lacewing, *Chrysoperla carnea* Stephens, and the minute pirate bug, *Orius insidiosus* (Say) (Sparks et al. 1966, Gordon 1985, Jarvis & Guthrie 1987). These three species utilize corn plants in several ways: as a food source by feeding on pollen, a substrate for oviposition, and as a host to other insect-prey including corn leaf aphids and thrips (Smith 1965, Hodek 1973, Isenhour & Yeargan 1981, Andow 1990, Coll & Bottrell 1991).

Recently field corn has been genetically engineered to resist *O. nubilalis* larval feeding utilizing genes from *Bacillus thuringiensis* subspecies *kurstaki* (Koziel et al. 1993). To produce adequate protein concentrations within a corn plant, the *cry1Ab* gene encoding the protein from *Bt* had to be modified. Additionally, the protein is expressed throughout the plant until physiological plant maturity. Adverse effects of *Bt* corn on insect predators may arise as a result of several factors: 1) high concentration of *Bt* protein in corn pollen, 2) modifications of *cry1Ab* gene producing several protein variations in different hybrids, and 3) *Bt* protein being expressed for extended periods of time compared to *Bt* spray application (Jepson et al. 1994).

Relatively few studies have assessed the effects of *Bt* sprays or the introduction of transgenic *Bt* plants on nontarget natural enemies. In one study, M-One, a *Bt* microbial spray used to control Colorado potato beetle, *Leptinotarsa decemlineata,* decreased the predation rate of *C. maculata* (Giroux et al. 1994). Conversely, using transgenic plants, Johnson and Gould (1992) studied parasitism of *Heliothis virescens* (F.) on transgenic tobacco and reported that low levels of *Bt* in transgenic tobacco appears to be compatible with two larval parasitoids (*Campoletis sonorensis* (Cameron) and *Cardiochiles nigriceps* Viereck) in suppressing *H. virescens.* In addition, Sims (1995) found no adverse effects of transgenic *Bt* cotton (Cry1Ac) on several beneficial insects including: *Hippodamia convergens* Guerin-Meneville, *Apis mellifera* L., *Nasonia vitiripennis* (Walker), and *C. carnea.* With *Bt* microbial sprays and *Bt* expression

in transgenic plants affecting natural enemies differently, it is not known what effect transgenic *Bt* corn plants expressing high levels of protein may have on predators. No studies have been conducted evaluating effects of transgenic *Bt* pollen on the development and survival of insect predators or the potential effect transgenic corn plants may have on natural enemies in the field.

The objectives of this study were to 1) determine effects of transgenic *Bt* corn pollen on the preimaginal development and survival of *C. maculata, C. carnea,* and *O. insidiosus,* and 2) compare the temporal occurrence and abundance of insect predators on transgenic *Bt* corn and non*Bt* corn plants.

Materials and Methods

Laboratory Studies

Pollen. Pollen was collected from field-grown transgenic *Bt,* and non*Bt* corn plants of the same Ciba Seeds hybrid (Research Triangle Park, NC). The transgenic *Bt* corn plants originated from hybrid cross CG00554 x CG00526Bt (Event 176) hemizygous for a synthetic *cry1Ab* gene derived from *Bacillus thuringiensis* subspecies *kurstaki* (Koziel et al. 1993). Expression of the *cry1Ab* gene in the 176 transformation is driven by two promoters, a phosphoenolpyruvate carboxylase promoter and a pollen-specific promoter (Koziel et al. 1993).

Pollen was collected from July 6 through July 11, 1994 by placing tassel bags (Midco Enterprises, Inc., Des Moines, IA) over tassels and stapling them closed.

Tassels and bags were cut off and pollen inside the bags was sifted through a 200 micron mesh screen to remove anthers and contaminants. Pollen was dried for 24 hours at approximately 23°C, poured into glass vials and placed in a freezer at -20°C.

Pollen was sent to Ciba Seeds Agricultural Biotechnology Research Center (Research Triangle Park, NC) at the beginning of each laboratory study to determine *Bt* protein (Cry1Ab) concentrations using enzyme-linked immunosorbant assays (ELISA) (Koziel et al. 1993). Concentrations of Cry1Ab protein in the pollen were between 2.57 and 2.94 µg/g dry weight for all experiments. In a 48 hour bioassay, these levels would be expected to cause between 60 and 70% mortality of first instar *O. nubilalis* (Laura Privalle, Ciba Seeds, personal communication).

Coleomegilla maculata. The procedures to examine the influence of transgenic *Bt* pollen on development and survival was based on methodology from Smith (1960), Obrycki and Tauber (1978), and Pilcher and Obrycki (1994). Adults were collected in the Ames, IA area during November, 1994. Pairs were placed in 1/2- pint paper cages (Neptune Paper Products, Inc., Jersey City, NJ) and maintained under a photoperiod of 16:8 (L:D), 24 ± 1°C, and 30-50% relative humidity. Each pair was provided a standard diet of water, a Wheast® (Qualcepts Nutrients, Minneapolis, MN) -honey [1:1] mixture, and a daily supply of green peach aphids *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and pea aphids *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Coleomegilla*

maculata eggs were collected from mating pairs and placed at 26°C, L:D 16:8 (Ormord 1995). Each of three treatment groups received larvae from 11 female sources.

At hatching, first instars were systematically placed into one of three groups within one of three diets: *A. pisum*, non*Bt* pollen, or transgenic *Bt* pollen. Approximately equal numbers from each *C. maculata* female were started on each treatment diet. The non*Bt* pollen served as a check that pollen alone was a suitable food for larval development. *Acyrthosiphon pisum* provided an optimum diet to verify that experimental conditions were suitable for larval development. Each diet group contained 45 individuals reared individually in 30 dram glass vials (19 x 65 mm; Kimble Products). Larvae were reared in a Percival table top incubator at 26°C, 16:8 L:D.

Three *A. pisum* were provided daily to each larva in the aphid treatment group. Pollen amounts fed to *C. maculata* larvae were based on Pilcher and Obrycki (1994) who showed that *C. maculata* larvae successfully completed development on corn pollen. First and second instars were given 0.015 g of either non*Bt* or transgenic *Bt* pollen. Third instars received 0.021 g and fourth instars, 0.03 g of pollen. Following completion of the first instar, individuals were given one *A. pisum* at the beginning of the second, third, and fourth instar stages. Thus, an individual reared on pollen received 3 *A. pisum* during its larval developmental period. This pollen-aphid combination was used because survival of *C. maculata* larvae reared solely on pollen was less than 48% (Pilcher

& Obrycki 1994). In addition, *C. maculata* larvae cannot complete development on only 3 *A. pisum* (Ormord 1995). Water was provided to all individuals by placing a soaked 1 cm piece of dental wick in the vials.

Larvae were checked daily for ecdysis and mortality; preimaginal survival and developmental times were recorded for each larval stage. Adults were frozen, sexed and weighed one day after eclosion.

Orius insidiosus. Adults were collected from Kossuth and Story Counties in Iowa during summer and fall, 1994. Approximately 100 adults were evenly divided among five 1/2- pint paper cages and maintained at 26 ± 1°C, 16:8 L:D, 30-50% R.H. Adults were provided a daily supply of *O. nubilalis* egg masses and every two days received a fresh green bean, *Phaseolus vulgaris* L., as an egg-laying substrate (Andow 1990, Castañé & Zalom 1994). Beans were removed, placed into a 1/2-pint container and observed daily for 1st stage nymphs. Individual nymphs were placed into plastic seal tight dishes (50 x 9 mm, Fisher Scientific, Inc., Pittsburgh, PA). A 1 cm dia. opening at the top of each dish was covered with organdy mesh. Each nymph was given 1 cm of soaked dental wick as a water supply. Forty-five nymphs were placed into each of three treatments, transgenic *Bt* pollen, non*Bt* pollen, or *O. nubilalis* eggs. To avoid mold growth, fresh pollen (0.015 g) was provided to each nymph every 2 to 4 days. Fresh *O. nubilalis* egg masses were provided every 3 to 4 days.

Nymphs were checked daily for mortality. Upon completing preimaginal development, sex was determined, total developmental times were recorded and percentage survival was calculated.

Chrysoperla carnea. Two laboratory studies were conducted measuring effects of transgenic *Bt* pollen on development and survival of C. *carnea.* *Chrysoperla carnea* eggs were obtained from Rincon-Vitova Insectaries, Inc., Ventura, California, on 17 April and 1 June, 1995. Eggs were maintained at 24 ± 1°C, 16:8 L:D, 30-50% R.H.

In the first study, eggs within 1 day of hatching were placed into individual 30 dram glass vials containing one of three diets: an optimal diet of *Sitotroga cerealella* (Olivier) eggs (Tauber & Tauber 1983) (Rincon-Vitova Insectaries, Inc., Ventura, CA), non*Bt* pollen or transgenic *Bt* pollen. In this study, three groups of 15 larvae were reared on each diet (45 individuals per diet).

Low survival of *C. carnea* larvae on either type of pollen in the first study, necessitated a second study with the following modifications. In the second study, first instars were placed into vials containing pollen and there were two treatments, non*Bt* pollen and transgenic *Bt* pollen. The number of larvae on each treatment was doubled from 45 to 90. First instar *C. carnea* on either pollen received 0.015 g pollen the first 24 hrs and 1 drop of water applied to the side of the vial. After 24 hrs, larvae were given *S. cerealella* eggs. Following ecdysis, second instars were moved to a new vial containing 0.015 g pollen for 24 hours, and then provided *S. cerealella* eggs (0.003 g). Third instars were treated in a

similar manner. Thus, each larva received only pollen as a food source for at least 72 hours. Finally, the amount of *S. cerealella* eggs provided to larvae was increased from 0.0005 g in the first study to 0.003 g in the second study for each stadium.

Chrysoperla carnea larvae were checked daily for ecdysis and survival. Preimaginal developmental times were recorded for each larval stage. Survival was based upon the percentage of individuals completing preimaginal development. Adults were frozen and sexed.

Voucher specimens of *C. maculata, O. insidiosus,* and *C. carnea* are deposited in the Iowa State University Insect Collection, Department of Entomology, Iowa State University, Ames, IA 50011.

Statistical Analyses. For each predatory species, developmental data was analyzed by separately examining individuals that successfully completed development from those individuals that died. This was done to examine developmental delays and stadium-specific mortality which may have been caused by *Bt* protein in the pollen. Quantifying the time before death and the life stage when death occurred is important when evaluating effects of *Bt* toxins. Higher mortality occurring during earlier stages of development was used in this study to indicate a sensitivity to the *Bt* toxin.

Developmental data, survival, and adult weights were analyzed using ANOVA, for *C. maculata, O. insidiosus* (total developmental time and survival), and *C. carnea* (preimaginal developmental times and survival). Proportion

survival was arcsine transformed before analysis. Means for all measurements were separated using multiple comparisons among diet treatments using Student's *t*-test (JMP 3.1, SAS Institute Inc., Cary, NC 1995).

Field Study

Predator Survey. The temporal occurrence and abundance of insect predators on transgenic *Bt* corn and non*Bt* corn was assessed in 1994 and 1995. In 1994, transgenic *Bt* and non*Bt* seed (Ciba hybrid cross CG00554 x CG00526) was planted in four row plots, 3 x 7.6 m (10 x 25 ft) in a randomized complete block design at the Insectary field plot, Iowa State University, Ames, IA. In 1995, transgenic *Bt* and non*Bt* seed (Monsanto hybrid MON810) was planted in four row plots, 3 x 15.2 m (10 x 50 ft) in a randomized complete block design at Iowa State University's Woodruff farm 2 km southwest of Ames, IA. Rows each year were planted with a 0.76 m spacing between rows and 0.23 m spacing between plants within a row. Plots were replicated three times.

Three times each year, all life stages of predatory species and *O. nubilalis* egg masses were counted on corn plants at three times: before tasseling (July 7, 1994; August 1, 1995), during pollen-shed (July 19, 1994; August 6, 1995), and after pollen- shed (August 6, 1994; August 18, 1995). Each year, 18 plants were marked (6 plants in each of 3 replications) in each treatment and inspected on each survey date. Whole plant counts were used to estimate predator populations by externally examining plants for coccinellid, chrysopid, anthocorid, and nabid eggs, immatures, and adults in addition to *O. nubilalis* egg masses (Coll &

Bottrell 1991). In 1995, Arachnids were also counted and identified to family. We also inspected the silks for predators within the ear tips.

Statistical Analysis. ANOVA was used to evaluate effects of the type of corn (*Bt* and non *Bt*), replication and sampling time. Means were separated using Student's *t*-test contrast comparisons (SAS Institue, Inc. 1995). Statistical comparisons between years were not made because different hybrids and locations were used.

Results

Coleomegilla maculata

Among individuals completing development, there were no differences among pollen and aphid diets during the the first three larval stadia (Table 1). However, prolonged development (3 days) of the 4th stadium was observed for individuals fed *A. pisum* ($P < 0.0001$). No differences were observed in pupal developmental time. The *A. pisum* group had longer total development time (23.6 days) compared to the pollen groups (20.7 and 21.2 days) ($P < 0.0001$, Table 1).

There were no differences between the sexes in time to complete development (Table 1). However, both sexes took less time to develop on either pollen compared to the *A. pisum* group (females: $P < 0.0001$, males: $P = 0.007$, Table 1). Females were heavier than males in all treatments ($F = 4.79$, d.f. = 1, 100, $P = 0.04$). Females and males reared on pollen were heavier than those reared on *A. pisum* aphids (females: $P < 0.0001$, males: $P < 0.0001$). There was a

trend for higher survival of *C. maculata* on transgenic *Bt* pollen (89 ± 2.2%) compared to non*Bt* pollen (69 ± 5.9%) and *A. pisum* (64 ± 12.4%), although this was not statistically significant (Fig. 1).

For individuals not completing development, there were no differences among the diets in the average time until death ($P = 0.83$, Table 1). Fewer larvae reared on transgenic *Bt* pollen died (N = 5) compared to those reared on non*Bt* pollen (N = 14) and *A. pisum* aphids (N = 16) (Table 1). No larvae reared on transgenic *Bt* pollen died until the 3rd instar (Fig. 2; Table 1).

Orius insidiosus

Significant differences were observed in total developmental time ($P < 0.0001$, Table 2). Females and males reared on pollen developed slower than those reared on *O. nubilalis* eggs (females: $P < 0.0001$, males: $P = 0.0013$). However, there were no differences in developmental time between the two pollen treatments (Table 2). There were no differences in survival among nymphs fed transgenic *Bt* pollen (63 ± 12%), non*Bt* pollen (44 ± 10.2%), or *O. nubilalis* eggs (57 ± 11.5%) (Fig. 3). Nymphs that died lived 14.2 ± 1.7 days on *Bt* pollen and 16.5 ± 1.4 days on non*Bt* pollen, whereas those individuals that died when fed *O. nubilalis* eggs lived 9.2 ± 1.6 days ($P = 0.0051$, Table 2).

Chrysoperla carnea

Individuals reared on pollen averaged nearly 15 days longer to complete development than those reared on *S. cerealella* eggs in the first study ($P < 0.0001$, Table 3). The delay in developmental times on pollen occurred during each

developmental stadium (1st - 3rd instar: $P < 0.0001$, Table 3). Only one female completed development on pollen. In this study, a significantly higher percentage of individuals reared on *S. cerealella* eggs (68 ± 6.7%) survived compared with either pollen (22 ± 9.7%) (Fig. 4).

Individuals not completing development showed significant differences in larval developmental times (Table 3). Development of 1st, 2nd, and 3rd instars was slower on pollen compared to those reared on *S. cerealella* eggs (first, $P = 0.02$; second and third, $P < 0.0001$). Individuals that died on non *Bt* pollen survived an average of 23.3 ± 2.3 days which is significantly longer than those reared on *Bt* pollen (16.4 ± 2.3) and *S. cerealella* eggs (8.6 ± 3.6) ($P = 0.003$, Table 3). Eighteen *C. carnea* reared on *Bt* pollen died as first instars compared to 7 deaths on either non *Bt* pollen or *S. cerealella* eggs (Fig. 5). A majority of the individuals not completing development reared on non *Bt* pollen successfully reached the 3rd instar, whereas the majority of individuals reared on *Bt* pollen or *S. cerealella* eggs successfully reached the 2nd instar.

In the second study, 49 ± 4% survival was observed on both pollen/*S. cerealella* diets (Fig. 4). Total development time averaged 22 ± 0.2 days for both pollen treatments (Table 4). Thirty-four and 33 individuals died as first instars on *Bt* and non *Bt* pollen, respectively (Fig. 6).

Predator Survey

There were no significant differences observed in the numbers of predators colonizing either non*Bt* or transgenic *Bt* corn in 1994 and 1995 (Tables

5, 6). In 1994, there was a significant treatment x sampling date interaction for coccinellid eggs (Table 6); there were more eggs laid on *Bt* corn plants before pollen shed than during pollen shed (Table 5). There were significantly more coccinellid larvae present during pollen shed compared to before and after pollen shed ($P = 0.01$, Tables 5, 6). Significantly more *C. maculata* adults ($P = 0.03$) were observed after pollen shed. *Hippodamia convergens* adults were observed on plants before pollen shed, but were not found during and after pollen shed. There were also significantly more *O. insidiosus* nymphs and adults found before and after pollen shed (Tables 5, 6). One chrysopid larva and one adult was counted. In addition, fewer than 15 *Nabis* adults were counted. In 1994, *Ostrinia nubilalis* egg masses were found on plants following pollen shed; none were found before or during pollen shed (Tables 5, 6).

Almost 70% fewer coccinellid eggs, larvae, and adults were found in 1995 (Table 5). Similar to 1994, more *C. maculata* adults were found on both types of corn following pollen shed (Tables 5, 6). No *H. convergens* adults were observed, however, six more chrysopid larvae and adults were encountered in 1995 (Table 5). There were significantly more chrysopid eggs found during pollen shed than before or after pollen shed (Tables 5, 6). More *O. insidiosus* nymphs and adults were observed following pollen shed compared to the two earlier samples (Tables 5, 6). Higher numbers of Arachnids were found after pollen shed compared to before and during pollen shed (Tables 5, 6). The number of *O. nubilalis* egg masses following pollen shed averaged close to one per plant and

was significantly higher than the number found before or during pollen shed (Tables 5, 6).

Discussion

There are no acute adverse effects of transgenic *Bt* corn pollen expressing the Cry1Ab protein on three predatory species tested: *C. maculata, O. insidiosus,* and *C. carnea.* Results supporting this conclusion were obtained by measuring the following developmental factors: survival, adult weight, and developmental time. One possible effect of transgenic *Bt* corn pollen might have been a delay in development, which would indicate a difference in the amount of food intake and/or a physiological response due to *Bt* protein consumption. Developmental time analyses for each species were performed separately for those individuals that completed development and those that died before completing development. Recording the average time until death in addition to stadium-specific mortality could indicate potential *Bt* effects.

Mortality occurs in an insect if a lethal dose of *Bt* protein is ingested before paralysis of the gut occurs which causes an insect to stop feeding (Gill et al. 1992, Bryant 1994). Early instars are generally subject to higher mortality because less *Bt* protein is required to receive a lethal dose. Thus, completion of early larval stadia would indicate a lower probability of any effect from *Bt* toxin activity. Activity of *Bt* is dependent on two factors: 1) structure of *Bt* and 2) the presence of

receptors in the insect midgut (Höfte & Whitely 1989). These two factors warrant *Bt* to be highly specific against certain insects.

The Cry1Ab protein used in *Bt* corn is lepidopteran-specific with high activity against *O. nubilalis* (Beegle & Yamamoto 1992, Gill et al. 1992, Denolf et al. 1993, and Cooper 1994). This would suggest that transgenic *Bt* corn would only affect lepidopteran species. However, the gene for the *Bt* protein was modified to increase expression levels in corn plants (Koziel et al. 1993). Because these modifications alter the level and form of *Bt* expression (plant), the potential exists for transgenic *Bt* corn plants to affect non-lepidopteran insects. In addition to the protein modifications, predators may not feed on pollen containing *Bt* protein because the suitability of pollen as a food source may have been altered. These potential effects requires assessment of this novel expression system of *Bt* protein in transgenic plants given the exposure to many natural enemies (Jepson et al. 1994). Therefore, predators were examined in three separate orders: Coleoptera, Hemiptera, and Neuroptera.

We observed no adverse effects (mortality or delayed development) of the *Bt* pollen on survival, developmental time, and adult weight of *C. maculata.* Transgenic *Bt* pollen appeared to be as suitable as non *Bt* pollen for food consumption. Similar results were found with *C. maculata* when Giroux et al. (1994) soaked pollen in a coleopteran-specific *Bt* microbial insecticide (M-One, *Bacillus thuringiensis* subsp. *san diego*). In addition, Melin & Cozzi (1990) reported no effects on *C. maculata* populations in a 2-year field study where fields

were sprayed with lepidopteran-specific *Bt* microbial sprays. Even though these two studies examined *Bt* sprays and the current study examines the novel expression of *Bt* in pollen, they support our conclusion that *C. maculata* is not adversely affected by transgenic corn pollen containing a lepidopteran-specific *Bt* protein.

There are no indications of detrimental effects caused by transgenic *Bt* pollen on *O. insidiosus*. Survival was similar for individuals reared on either type of pollen and *O. nubilalis* eggs. However, nymphs reared on either pollen took significantly longer to complete development compared to nymphs fed *O. nubilalis* eggs. It has previously been observed that *O. insidiosus* fed arthropods developed faster than those provided with plant tissue (Kiman & Yeargan 1985). Kiman and Yeargan (1985) also found survival of *O. insidiosus* reared on *Acer* spp. pollen to be greater than 90%. The lower survival in this study may be due to use of corn pollen. In a different study, the average preimaginal developmental time for *O. insidiosus* when fed *Heliothis virescens* eggs was 14.9 days at 24°C (Isenhour & Yeargan 1981). In our study, slower development of *O. insidiosus* was observed where nymphs reared on *O. nubilalis* eggs completed development in 18.1 days at 26°C. However, there were no differences in developmental times for individuals reared on either pollen diet.

Two studies were conducted to determine possible effects of *Bt* pollen on *C. carnea*. In both studies, *C. carnea* was exposed to pollen under no-choice conditions for 24 hours following ecdysis before being supplied with a

supplement of *S. cerealella* eggs. It has been previously reported that *C. carnea* larvae are unable to survive for more than 24 hours without food (Principi & Canard 1984). Results from the first study indicated possible *Bt* pollen effects because 40% of the first instars died. However, our methods were reevaluated when the survival of individuals reared on either pollen was only 22%. First instars that died after one day possibly starved because they did not feed on the pollen or the pollen may not have been suitable to develop to the 2nd instar. In study #2, a larger supplement of *S. cerealella* eggs was provided. In addition, surviving larvae were observed feeding on the pollen during all developmental stadia. The low survival in both studies may be attributed to a missing dietary requirement in corn pollen because Niijima (1993) reported that no chrysopid larva molted to the second instar on diets lacking any of 10 required amino acids.

However, low survival may have also been attributed to the methods used. In the first study, eggs were placed into the vials before hatching. In the second study, eggs were allowed to hatch and then the larvae were transferred to the vials. In the second study, survival of individuals fed either pollen was 49%. In addition, there were no differences in development observed between pollen treatments in the second study. We conclude that there is no *Bt* pollen effect on *C. carnea* larval development.

Two other studies have been conducted on the nontarget effects of a transgenic organism on natural enemies. Two predators, *C. carnea* and *O. insidiosus* were not adversely affected by feeding on larvae of *H. virescens*

infected with a recombinant nuclear polyhedrosis virus (Heinz et al. 1994). This study evaluated how a genetically altered virus may impact these predators where our study evaluated the effects of a genetically altered bacterium expressed in plant tissue. Sims (1995) studied the effects of *Bt* Cry1Ac protein expressed in transgenic cotton on two insect predators, *H. convergens* and *C. carnea*. He found no detrimental effects on these predators, however, he did not use the plant parts for the study where we used corn pollen expressing the the *Bt* protein. There will be a continued need for such nontarget studies in the future.

In addition to laboratory studies, field studies were conducted to determine possible effects of *Bt* corn on predators. Generalist predators are common in corn and utilize pollen as a food source as well as a substrate for oviposition (Sparks et al. 1966, Jarvis & Guthrie 1987, Andow 1990, Coll & Bottrell 1991, 1992). Plant antibiosis (*Bt* corn) not only influences phytophagous species (pests), but can affect species over three or four trophic levels (Price 1981, Orr & Boethel 1986, Rice & Wilde 1989). Therefore, even though predators feeding on *Bt* pollen did not show developmental effects, *Bt* corn could alter predator behavior under field conditions.

There were no differences in the abundance of predators observed on transgenic *Bt* or non*Bt* corn, but differences were observed among sampling dates. In 1994, significantly different numbers of predators were observed between sampling dates for coccinellid larvae, *C. maculata* adults, *H. convergens* adults and *O. insidiosus* nymphs and adults. In 1995, significantly different

numbers were observed between sampling dates for *C. maculata* adults, chrysopid eggs, *O. insidiosus* nymphs and adults, and arachnids. Compared to 1994, predators were less abundant in 1995 on variety (MON810) at the ISU Woodruff farm, however, patterns can be observed for both years. For example, more *C. maculata* adults were present after pollen shed both years. In addition, the number of *O. insidiosus* present during pollen shed tended to be lower than before or after pollen shed both years. Both years differ from previous findings in which higher populations of *O. insidiosus* were observed when the ears had green silks, i.e. at pollen shed (Barber 1936, Dicke & Jarvis 1962). This would suggest that presence of pollen may have impacted the number of *O. insidiosus* present in these studies. However, previous studies looked at the abundance of all stages of *O. insidiosus* on random plants chosen from arbitrary field sites (Barber 1936, Dicke & Jarvis 1962). Therefore, the size of our plots may have been a factor because our plots were only 4 rows wide.

Transgenic *Bt* pollen may have an effect if we consider the number of buffer rows used between plots. Every plant used for sampling had only 1 buffer row between it and the next plot and the two middle rows of every plot was used. With four-row plots, given wind dispersal of pollen, *Bt* and non *Bt* pollen could completely mix and cover all rows within the plots. This movement of pollen could explain there being no significant difference in the number of predators between corn types, yet significant sampling date differences for several of the predator species were observed. If *Bt* and non *Bt* pollen mixed, *Bt* pollen could

possibly cover the entire plot. If predator numbers decreased in both *Bt* and non *Bt* corn counts, this decrease could be due to *Bt* pollen. This effect could also explain the treatment*sampling time interactions that were significant for coccinellid eggs in 1994 and coccinellid larvae in 1995. However, the ratio of *Bt* pollen to non *Bt* pollen in *Bt* corn plots should be relatively high and the opposite observed in non *Bt* plots. There are other plant characteristics that contribute to predator abundance besides pollen. Even though no differences in predator abundance were found between corn types in our study, future studies are needed on larger fields.

Methods for assessing the risk of transgenic plants have been outlined (Jepson et al. 1994, Kjellsson & Simonsen 1994). These methods need to be used to determine potential ecological impacts that may arise by using transgenic crops on a large-scale basis. In addition, laboratory developmental studies evaluating chronic and reproductive responses for more than one generation with insects that feed on transgenic *Bt* corn plants need to be completed.

Preservation of natural enemies associated with crop pests such as *O. nubilalis* is one of the most important tactics in modern integrated pest management programs (Metcalf & Luckmann 1992, Dent 1991). By studying the compatibility between transgenic plants and natural enemies under laboratory and field conditions, we will better understand what future pest management possibilities may be available. For example, one study has shown that low levels

of *Bt* in transgenic tobacco and natural enemies can work synergistically to reduce pest populations (Johnson & Gould 1992).

Our study shows that transgenic *Bt* corn pollen does not have acute toxic effects on three prominent predators found in corn. In addition, we observed that predators will use *Bt* corn as a source of food (pollen, aphids, etc.) and as a substrate for oviposition. These results may benefit future integrated pest management systems in utilizing biologcal control and host-plant resistance to combat corn pests.

Acknowledgments

We thank undergraduate workers J. Ostrem, C. Grutzmacher, T. Cortilet, and E. Butin for field and laboratory assistance. We thank Dr. Paul Hinz, Department of Statistics, Iowa State University for statistical advice. We also thank Rincon-Vitova Insectaries, Inc. for supplying *C. carnea* in addition to Jean Dyer, USDA-ARS, for supplying *O. nubilalis* eggs. This research was supported in part by Ciba Seeds Agricultural Biotechnology Group, Leopold Center for Sustainable Agriculture grant and Dupont Chemical in the form of a Student Scholar award. C.D.P. was supported by a research/extension assistantship from the Department of Entomology, Iowa State University. Journal Paper No. J-xxxxx of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 2755.

References Cited

Andow, D. A. 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482-486.

Barber, G. W. 1936. *Orius insidiosus* (Say), an important natural enemy of the corn ear worm. U. S. Dept. of Ag. Tech. Bull. No. 504: 1-24.

Barry, D. & L. L. Darrah. 1991. Effect of research on commercial hybrid maize resistance to European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 84: 1053-1059.

Beegle, C. C. & T. Yamamoto. 1992. Invitation paper (C.P. Alexander Fund): History of *Bacillus thuringiensis* Berliner research and development. Can. Entomol. 124: 587-616.

Bryant, J. E. 1994. Application strategies for *Bacillus thuringiensis*. Agriculture, Ecosystems, and Environment. 49: 65-75.

Castañé, C. & F. G. Zalom. 1994. Artificial oviposition substrate for rearing *Orius insidiosus* (Hemiptera: Anthocoridae). Biol. Cont. 4: 88-91.

Clausen, C. P. 1978. Introduced parasites and predators of arthropod pests and weeds: A world review. U.S. Dept. Agric. Agric. Handbook, 480.

Coll, M. & D. G. Bottrell. 1991. Microhabitat and resource selection of the European corn borer (Lepidoptera: Pyralidae) and its natural enemies in Maryland field corn. Environ. Entomol. 20: 526-533.

Coll, M. & D. G. Bottrell. 1992. Mortality of European corn borer larvae by natural enemies in different corn microhabitats. Biol. Con. 2: 95-103.

Cooper, D. 1994. *Bacillus thuringiensis* toxins and mode of action. Agriculture, Ecosystems, and Environment. 49: 21-26.

Denolf, P., S Jansens, M. Peferoen, D. Degheele & J. Van Rie. 1993. Two different *Bacillus thuringiensis* Delta-endotoxin receptors in the midgut brush border membrane of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). Appl. Environ. Microbiol. 59: 1828-1837.

Dent, D. 1991. Insect Pest Management. CAB International. Wallingford, UK.

Dicke, F. F. & J. L. Jarvis. 1962. The habits and seasonal abundance of *Orius insidiosus* (Say) (Hemiptera-Heteroptera: Anthocoridae) on corn. J. Kans. Entomol. Soc. 35: 339-344.

Gill, S. S., E. A. Cowles & P. V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37: 615-636.

Giroux, S., J. C. Côté, C. Vincent, P. Martel & D. Coderre. 1994. Bacteriological insecticide M-One effects on predation efficiency and mortality of adult *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae). J. Econ. Entomol. 87: 39-43.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. J. New York Entomol. Soc. 93: 1-912.

Heinz, K. M., B. G. McCutchen, R. Herrmann, M. P. Parrella & B. D. Hammock. 1995. Direct effects of recombinant nuclear polyhedrosis viruses on selected nontarget organisms. J. Econ. Entomol. 88: 259-264.

Hodek, I. 1973. Biology of Coccinellidae. Academia, Publishing House of the Academy of Sciences, The Hague, Prague.

Höfte, H. & H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiesis*. Microbio. Rev. 53: 242-255.

Isenhour, D. J. & K. V. Yeargan. 1981. Effect of temperature on the development of *Orius insidiosus*, with notes on laboratory rearing. Ann. Entomol. Soc. Am. 74: 114-116.

Jarvis, J. L. & W. D. Guthrie. 1987. Ecological studies of the European corn borer (Lepidoptera: Pyralidae) in Boone county, Iowa. Environ. Entomol. 16: 50-58.

Jepson, P. C., B. A. Croft & G. E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3: 81-89.

Johnson, M. T. & F. Gould. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *Heliothis virescens* (Lepidoptera: Noctuidae) in tobacco. Environ. Entomol. 21: 586-597.

Kiman, Z. B. & K. V. Yeargan. 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. Ann. Entomol. Soc. Am. 78: 464-467.

Kjellsson, G. & V. Simonsen. 1994. Methods for risk assessment of transgenic plants. Birkhäuser Verlag, Basel, Switzerland.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright & S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio/Technology 11: 194-200.

Lewis, L. C. & L. A. Bing. 1991. *Bacillus thuringiensis* and *Beauveria bassiana* (Balsamo) Vuillimen for European corn borer control: program for immediate and season-long suppression. Can. Entomol. 123: 387-393.

Melin, B. E. & E. M. Cozzi 1990. Safety to nontarget invertebrates of lepidopteran strains of *Bacillus thuringiensis* and their ß-exotoxins pp. 149-168. *In* M. Laird, L. Lacey, & E Davidson's [eds.] Safety of microbial insecticides. CRC Press, Boca Raton, FL.

Metcalf, R. L. & W. H. Luckmann. 1992. Introduction to insect pest management, 3rd ed., John Wiley & Sons, New York, New York.

Niijima, K. 1993. Nutritional studies on an aphidophagous chrysopid, *Chrysopa septempunctata* Wesmael (Neuroptera: Chrysopidae) II. Amino acid requirement for larval development. Appl. Entomol. Zool. 28: 81-87.

Obrycki, J. J. & M. J. Tauber. 1978. Thermal requirements for development of *Coleomegilla maculata* (Coleoptera: Coccinellidae) and its parasite *Perilitus coccinellae* (Hymenoptera: Braconidae). Can. Entomol. 110: 407-412.

Ormord, A. M. 1995. Interactions between larval *Coccinella septempunctata* L. and *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae). M.S. thesis. Iowa State University, Ames.

Orr, D. B. & D. J. Boethel. 1986. Influence of plant antibiosis through four trophic levels. Oecologia. 70: 242-249.

Pilcher, C. D. & J. J. Obrycki. 1994. Feeding, development and survival of the lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae) on transgenic *B.t.* corn pollen, pp. 261-263. *In* Proceedings, The Integrated Crop Management Conference, 30 November - 1 December, 1994. Iowa State University Extension, Ames.

Price, P.W. 1981. Semiochemicals in Evolutionary Time, pp. 251-259. *In* Nordlund, D.A., R.L. Jones & W.J. Lewis [eds.], Semiochemicals: Their role in pest control. John Wiley & Sons Ltd., New York, New York.

Principi, M. M. & M. Canard. 1984. Feeding habits, pp. 76-91. *In* M. Canard, Y. Séméria & T. R. New [eds.], Biology of Chrysopidae. Kluwer Academic Publishers, Hingham, MA.

Rice, M.E. 1994. Aerial application of insecticides for control of second generation European corn borer: 1991-1993. Arthropod management tests 19: 204-206.

Rice, M.E. & G. E. Wilde 1989. Antibiosis effect of sorghum on the convergent lady beetle (Coleoptera: Coccinellidae), a third-trophic level predator of the greenbug (Homoptera: Aphididae). J. Econ. Entomol. 82: 570-573.

SAS Institute, Inc. 1995. JMP® statistics and graphics guide, version 3.1. SAS Institute, Inc. Cary, North Carolina, U.S.A.

Showers, W. B., J. F. Witkowski, C. E. Mason, D. D. Calvin, R. A. Higgins & G. P. Dively. 1992. European corn borer - development and management. Iowa State University. North Central Regional Extension Publ. 327.

Sims, S. R. 1995. *Bacillus thuringiensis* var. *kurstaki* (Cry1Ac) protein expressed in transgenic cotton: Effects on beneficial and other non-target insects. Southwestern Entomologist. 20: 493-500.

Smith, B. C. 1960. A technique for rearing coccinellid beetles on dry foods, and influence of various pollens on the development of *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae). Can. J. Zool. 38: 1047-1049.

Smith, B. C. 1965. Growth and development of coccinellid larvae on dry foods (Coleoptera: Coccinellidae). Can. Entomol. 97: 760-768.

Sparks, A. N., H. C. Chiang, C. C. Burkhardt, M.L. Fairchild & G. T. Weekman. 1966. Evaluation of the influence of predation on corn borer populations. J. Econ. Entomol. 59: 104-107.

Tauber, M. J. & C. A. Tauber. 1983. Life history traits of *Chrysopa carnea* and *Chrysopa rufilabris* (Neuroptera: Chrysopidae): influence of humidity. Ann. Entomol. Soc. Am. 76: 282-285.

Table 1. Developmental responses ($\bar{x}$ ± SEM) of *C. maculata* feeding on transgenic *Bt* pollen, non*Bt* pollen, or *A. pisum* 26 ± 1ºC, 16:8 (L:D).

Developmental stadia	Isogenic non*Bt* pollen	Transgenic *Bt* pollen	*Acyrthosiphon pisum*	*F*	*P*
Individuals completing development ($\bar{x}$ days ± SEM)[1] d.f. = 2, 97					
1st instar	3.0 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	1.8	0.18
2nd instar	2.5 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	1.2	0.32
3rd instar	3.3 ± 0.1	3.3 ± 0.1	3.0 ± 0.2	2.0	0.14
4th instar	5.4 ± 0.2**a**	5.4 ± 0.2**a**	8.3 ± 0.2**b**	59.4	**<0.001**
pupa	3.6 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	0.4	0.67
Total (N)	20.7 ± 0.3 (31)**a**	21.2 ± 0.3 (40)**a**	23.6 ± 0.4 (29)**b**	18.7	**<0.001**
Developmental time by sex ($\bar{x}$ days ± SEM) (N) female d.f. = 2, 63, male d.f. = 2, 31					
females	20.9 ± 0.4 (20)**a**	21.2 ± 0.3 (30)**a**	23.8 ± 0.5 (16)**b**	13.1	**<0.001**
males	20.3 ± 0.7 (11)**a**	21.1 ± 0.7 (10)**a**	23.3 ± 0.6 (13)**b**	5.9	**0.007**
Adult weight (g) female d.f. = 2, 63, male d.f. = 2, 31					
females	0.011 ± 0.0003**a**	0.011 ± 0.0003**a**	0.008 ± 0.0004**b**	17.6	**<0.001**
males	0.010 ± 0.0003**a**	0.010 ± 0.0003**a**	0.007 ± 0.0003**b**	23.5	**<0.001**
Stadium-specific development time - Individuals that failed to complete development ($\bar{x}$ days ± SEM) (N)[2]					
1st instar (2, 32)	3.4 ± 0.2 (14)	3.2 ± 0.4 (5)	3.2 ± 0.2 (16)	0.2	0.86
2nd instar (2, 30)	2.6 ± 0.2 (14)	3.4 ± 0.4 (5)	2.3 ± 0.2 (14)	3.2	0.06
3rd instar (2, 26)	3.7 ± 0.5 (11)	4.0 ± 0.7 (5)	3.2 ± 0.4 (13)	0.7	0.49
4th instar (2, 18)	4.4 ± 0.8 (8)	2.5 ± 1.7 (2)	6.0 ± 0.7 (11)	2.4	0.12
pupa	4.0 (2)	-	6.0 (1)	-	-
$\bar{x}$ days ± SEM until death (N) d.f. = 2, 32					
	15.1 ± 1.2 (14)	14.6 ± 2.1 (5)	15.9 ± 1.2 (16)	0.2	0.83

[1] Bold letters show significant differences among treatments.

[2] N is the number of individuals that completed each stadium, but died before reaching adult stage. Degrees of freedom are given in far left column.

Table 2. Developmental responses ($\bar{x}$ ± SEM) of *O. insidiosus* feeding on transgenic *Bt* pollen, non*Bt* pollen, or *O. nubilalis* eggs 26 ± 1ºC, 16:8 (L:D).

	Isogenic non*Bt* pollen	Transgenic *Bt* pollen	*Ostrinia nubilalis* eggs	*F*	*P*
Individuals completing development ($\bar{x}$ days ± SEM) (N)[1]					
Total (2, 66)	21.9 ± 0.4 (19)**a**	22.1 ± 0.4 (28)**a**	18.1 ± 0.4 (22)**b**	30.1	**<0.001**
females (2, 32)	23.0 ± 0.5 (7)**a**	22.4 ± 0.5 (16)**a**	18.4 ± 0.4 (12)**b**	25.8	**<0.001**
males (2, 31)	21.3 ± 0.4 (12)**a**	21.6 ± 0.7 (12)**a**	17.7 ± 0.7 (10)**b**	10.6	**<0.001**
Individuals not completing development					
Average time (days) alive for individuals that failed to reach adult stage (N)					
d.f. = 2, 53					
	16.5 ± 1.4 (23)**a**	14.2 ± 1.7 (16)**a**	9.2 ± 1.6 (17)**b**	5.9	**0.01**

[1] Bold letters show significant differences among treatments. Degrees of freedom are given in far left column.

Table 3. Developmental responses ($\bar{x}$ ± SEM) of *C. carnea* fed transgenic *Bt* pollen, non*Bt* pollen, or *S. cerealella* eggs 24 ± 1°C, 16:8 (L:D).

Developmental stadia	Isogenic non*Bt* pollen	Transgenic *Bt* pollen	*Sitotroga cerealella* eggs	*F*	*P*
Individuals completing development ($\bar{x}$ days ± SEM)[1] d.f. = 2, 47					
1st instar	6.7 ± 0.6**a**	6.0 ± 0.5**a**	3.7 ± 0.3**b**	14.0	**<0.001**
2nd instar	6.7 ± 0.4**a**	6.6 ± 0.4**a**	3.0 ± 0.2**b**	43.8	**<0.001**
3rd instar	12.6 ± 0.4**a**	13.5 ± 0.4**a**	5.1 ± 0.2**b**	242	**<0.001**
pupa	9 ± 0.2**a**	9 ± 0.2**a**	8.5 ± 0.1**b**	3.5	**0.038**
Total (N)	35 ± .7 (10)**a**	35.1 ± 0.7 (10)**a**	20.3 ± 0.4 (30)**b**	246	**<0.001**
Developmental time x sex (days) (N) female d.f. = 1, 8, male d.f. = 2, 37					
females	-	26 ± 0.5 (1)**a**	20.4 ± 0.2 (9)**b**	100	**<0.001**
males	35 ± 0.6 (10)**a**	36.1 ± 0.7 (9)**a**	20.2 ± 0.4 (21)**b**	280	**<0.001**
Stadium-specific development time - Individuals that failed to complete development ($\bar{x}$ days ± SEM) (N)[2]					
1st instar (2, 81)	4.3 ± 0.3 (35)**a**	3.6 ± 0.3 (35)**ab**	2.6 ± 0.5 (14)**b**	4.1	**0.021**
2nd instar (2, 49)	6.9 ± 0.3 (28)**a**	6.7 ± 0.4 (17)**a**	2.6 ± 0.7 (7)**b**	18.1	**<0.001**
3rd instar (2, 48)	11.8 ± 0.5 (28)**b**	13.6 ± 0.7 (17)**a**	5.3 ± 1.2 (6)**c**	18.8	**<0.001**
pupa (2, 27)	9.6 ± 0.2 (15)	9.3 ± 0.4 (11)	8.8 ± 0.3 (4)	1.2	0.306
$\bar{x}$ days ± SEM until death (N) d.f. = 2, 81					
	23.3 ± 2.3 (35)**a**	16.4 ± 2.3 (35)**b**	8.6 ± 3.6 (14)**b**	6.4	**0.003**

[1] Bold letters show significant differences between treatments. Individuals received pollen for first 24 hours of the 1st, 2nd, and 3rd stadia. Following this each individual received a mixture of pollen and *S. cerealella* eggs to complete each stadium.

[2] N is the number of individuals that completed each stadium, but died before reaching adult stage. Degrees of freedom are given after each life stage.

Table 4. Developmental responses ($\bar{x}$ ± SEM) of *C. carnea* fed transgenic *Bt* pollen and *S. cerealella* eggs, or non*Bt* pollen and *S. cerealella* eggs 24 ± 1°C, 16:8 (L:D).

Developmental stadia	Isogenic non*Bt* pollen	Transgenic *Bt* pollen	*F*	*P*
	Individuals completing development ($\bar{x}$ days ± SEM)[1]			
	d.f. = 1, 84			
1st instar	5.6 ± 0.1	5.6 ± 0.1	0.12	0.73
2nd instar	3.4 ± 0.1	3.3 ± 0.1	0.45	0.50
3rd instar	4.3 ± 0.2	4.5 ± 0.2	0.42	0.52
pupa	8.7 ± 0.1	8.7 ± 0.1	0.03	0.85
Total (N)	22.1 ± 0.2 (43)	22.2 ± 0.2 (43)	0.02	0.89
	Developmental time x sex (days) (N)			
	female d.f. = 1, 35, male d.f. = 1, 47			
females	22.8 ± 0.4 (14)	22.7 ± 0.3 (23)	0.03	0.87
males	21.8 ± 0.3 (29)	21.6 ± 0.3 (20)	0.3	0.56
Stadium-specific development time - Individuals that failed to complete development ($\bar{x}$ days ± SEM) (N)[2]				
1st instar (1, 87)	2.8 ± 0.3 (45)	3.0 ± 0.3 (44)	0.4	0.51
2nd instar (1, 20)	3.6 ± 0.2 (12)	3.2 ± 0.2 (10)	2.5	0.13
3rd instar (1, 17)	4.9 ± 0.6 (11)	4.5 ± 0.7 (8)	0.2	0.67
pupa (1, 14)	9.0 ± 0.5 (8)	10.4 ± 0.5 (8)	3.5	0.08
	$\bar{x}$ days ± SEM until death (N)			
	d.f. = 1, 87			
	6.5 ± 1.3 (45)	6.5 ± 1.3 (44)	0	0.98

[1] Individuals received pollen for first 24 hours of the 1st, 2nd, and 3rd stadia. Following this, each individual received a mixture of pollen and *S. cerealella* eggs to complete each stadium.

[2] N is the number of individuals that completed each stadium, but died before reaching adult stage. Degrees of freedom are given after each life stage.

Table 5. Mean (± SEM) number of predators present per plant on each sampling date (36 plants examined each date - 18 *Bt* corn, 18 non *Bt* corn): before pollen shed (before) (July 7, 1994, August 1, 1995); pollen shed (shed) (July 19, 1994, August 6, 1995); after pollen shed (after) (August 6, 1994, August 18, 1995). If ANOVA was significant, means were separated using Students *t*-test multiple comparisons. Mean differences among sampling dates are indicated by different letters. See Table 6 for statistical comparisons.

	1994						1995					
Predator and stages found	Isogenic non*Bt* corn			Transgenic *Bt* corn			Isogenic non*Bt* corn			Transgenic *Bt* corn		
	before	**shed**	**after**	**before**	**shed**	**after**	**before**	**shed**	**after**	**before**	**shed**	**after**
coccinellid eggs	.94 ± 0.7	1.5 ± 1.0	2.3 ± 1.3	5.6 ± 1.8	0.2 ± 0.2	1.9 ± 1.4	0	0	0	0.2 ± 0.2	0	0
coccinellid larvae	0.8 ± 0.5b	2.3 ± 0.8a	0.3 ± 0.1c	2.6 ± 1.2b	2.2 ± 0.8a	0.1 ± 0.1c	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.6 ± 0.4	0.6 ± 0.2	0
C. maculata adults	0.4 ± 0.2b	0.3 ± 0.1c	0.9 ± 0.4a	0.6 ± 0.2b	0.3 ± 0.2c	0.8 ± 0.2a	0.1 ± 0.1b	0.1 ± 0.1b	0.4 ± 0.2a	0b	0b	0.4 ± 0.1a
H. convergens adults	0.2 ± 0.1a	0b	0b	0.2± 0.1a	0b	0b	0	0	0	0	0	0
chrysopid eggs	2.7 ± 0.7	1.4 ± 0.4	1.5 ± 0.5	2.1 ± 0.4	1.7 ± 0.3	1.4 ± 0.3	1.4 ± 0.3b	2.6 ± 0.4a	1.2 ± 0.3b	1.2 ± 0.3b	3.1 ± 0.6a	2.4 ± 0.5b
chrysopid larvae	0	0.1 ± 0.1	0	0	0	0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0	0
chrysopid adults	0	0.1 ± 0.1	0	0	0	0	0	0	0.1 ± 0.1	0	0	0.1 ± 0.1
O. insidiosus nymphs and adults	2.6 ± 0.4a	0.8 ± 0.2b	2.1 ± 0.4a	1.9 ± 0.4a	0.9 ± 0.3b	2.4 ± 0.4a	0.8 ± 0.3b	0.6 ± 0.2b	1.4 ± 0.3a	0.7 ± 0.2b	1.1 ± 0.1b	2.3 ± 0.5a
Nabis adults	0.2 ± 0.1	0.1 ± 0.1	0	0.2 ± 0.1	0.2± 0.1	0.1 ± 0.1	0	0	0	0	0	0.1 ± 0.1
Arachnids	-	-	-	-	-	-	0.4 ± 0.2b	0.2 ± 0.1b	0.9 ± 0.2a	0.4 ± 0.2b	0.1 ± 0.1b	0.9 ± 0.2a
O. nubilalis egg masses	0b	0b	1.4 ± 0.3a	0b	0b	1.1 ± 0.3a	0.1 ± 0.1b	0.1 ± 0.1b	0.6 ± 0.2a	0.1 ± 0.1b	0.3 ± 0.1b	0.8 ± 0.2a

Table 6. ANOVA table for predator abundance and seasonal occurrence from Table 5. Values represent the effect tested under the source column. Significance is indicated in bold.

1994					1995				
Predator and stages found	Source[1]	d.f.[2]	*F*	*P*	Predator and stages found	Source[1]	d.f.[2]	*F*	*P*
coccinellid eggs	Treatment	1	1.02	0.31	coccinellid eggs	Treatment	1	1.0	0.32
	Rep	2	1.27	0.28		Rep	2	1.0	0.37
	Treatment*Rep	2	0.90	0.41		Treatment*Rep	2	1.0	0.37
	Sampling date	2	2.07	0.13		Sampling date	2	1.0	0.37
	Treatment*SD	2	3.67	**0.03**		Treatment*SD	2	1.0	0.37
coccinellid larvae	Treatment	1	0.78	0.37	coccinellid larvae	Treatment	1	0.98	0.32
	Rep	2	0.08	0.92		Rep	2	2.20	0.12
	Treatment*Rep	2	3.35	**0.04**		Treatment*Rep	2	2.66	0.07
	Sampling date	2	4.97	**0.01**		Sampling date	2	0.16	0.85
	Treatment*SD	2	1.31	0.27		Treatment*SD	2	3.32	**0.04**
C. maculata adults	Treatment	1	0.01	0.92	*C. maculata* adults	Treatment	1	0.23	0.63
	Rep	2	1.17	0.32		Rep	2	0.40	0.67
	Treatment*Rep	2	0.29	0.75		Treatment*Rep	2	1.08	0.34
	Sampling date	2	3.58	**0.03**		Sampling date	2	9.61	**0.0002**
	Treatment*SD	2	0.32	0.72		Treatment*SD	2	0.06	0.94
H. convergens adults	Treatment	1	-	-	chrysopid eggs	Treatment	1	2.27	0.14
	Rep	2	1.61	0.21		Rep	2	0.09	0.92
	Treatment*Rep	2	-	-		Treatment*Rep	2	1.46	0.24
	Sampling date	2	4.82	**0.01**		Sampling date	2	6.79	**0.0017**
	Treatment*SD	2	-	-		Treatment*SD	2	1.58	0.21

[1] SD = Sampling date

[2] Degrees of freedom for all error tests is 98.

Table 6 continued

chrysopid eggs	Treatment	1	0.24	0.63	*Chrysopa* larvae	Treatment	1	0.68	0.41
	Rep	2	0.71	0.50		Rep	2	0.51	0.61
	Treatment*Rep	2	6.46	**0.0023**		Treatment*Rep	2	0.17	0.84
	Sampling date	2	2.86	0.06		Sampling date	2	1.52	0.22
	Treatment*SD	2	0.50	0.61		Treatment*SD	2	0.17	0.84
chrysopid larvae	Treatment	1	1.0	0.32	chrysopid adults	Treatment	1	-	-
	Rep	2	1.0	0.37		Rep	2	0.50	0.61
	Treatment*Rep	2	1.0	0.37		Treatment*Rep	2	1.50	0.23
	Sampling date	2	1.0	0.37		Sampling date	2	2.00	0.14
	Treatment*SD	2	1.0	0.37		Treatment*SD	2	-	-
chrysopid adults	Treatment	1	1.0	0.32	O. insidiosus nymphs and adults	Treatment	1	2.78	0.10
	Rep	2	1.0	0.37		Rep	2	0.57	0.57
	Treatment*Rep	2	1.0	0.37		Treatment*Rep	2	1.19	0.31
	Sampling date	2	1.0	0.37		Sampling date	2	9.11	**0.0002**
	Treatment*SD	2	1.0	0.37		Treatment*SD	2	1.38	0.26
O. insidiosus nymphs and adults	Treatment	1	0.04	0.85	*Nabis* adults	Treatment	1	2.23	0.14
	Rep	2	3.18	**0.04**		Rep	2	2.23	0.11
	Treatment*Rep	2	1.02	0.36		Treatment*Rep	2	2.23	0.11
	Sampling date	2	9.54	**0.0002**		Sampling date	2	2.23	0.11
	Treatment*SD	2	1.18	0.31		Treatment*SD	2	2.23	0.11
Nabis adults	Treatment	1	0.28	0.60	*O. nubilalis* egg masses	Treatment	1	2.37	0.13
	Rep	2	0.28	0.76		Rep	2	1.37	0.26
	Treatment*Rep	2	1.11	0.33		Treatment*Rep	2	0.93	0.40
	Sampling date	2	2.15	0.12		Sampling date	2	13.8	**<0.001**
	Treatment*SD	2	0.49	0.62		Treatment*SD	2	0.26	0.77
O. nubilalis egg masses	Treatment	1	0.53	0.47	Arachnids	Treatment	1	0.30	0.58
	Rep	2	5.31	**0.01**		Rep	2	0.17	0.84
	Treatment*Rep	2	0.34	0.71		Treatment*Rep	2	0.25	0.78
	Sampling date	2	42.6	**<0.001**		Sampling date	2	10.7	**0.0001**
	Treatment*SD	2	0.53	0.59		Treatment*SD	2	0.02	0.98

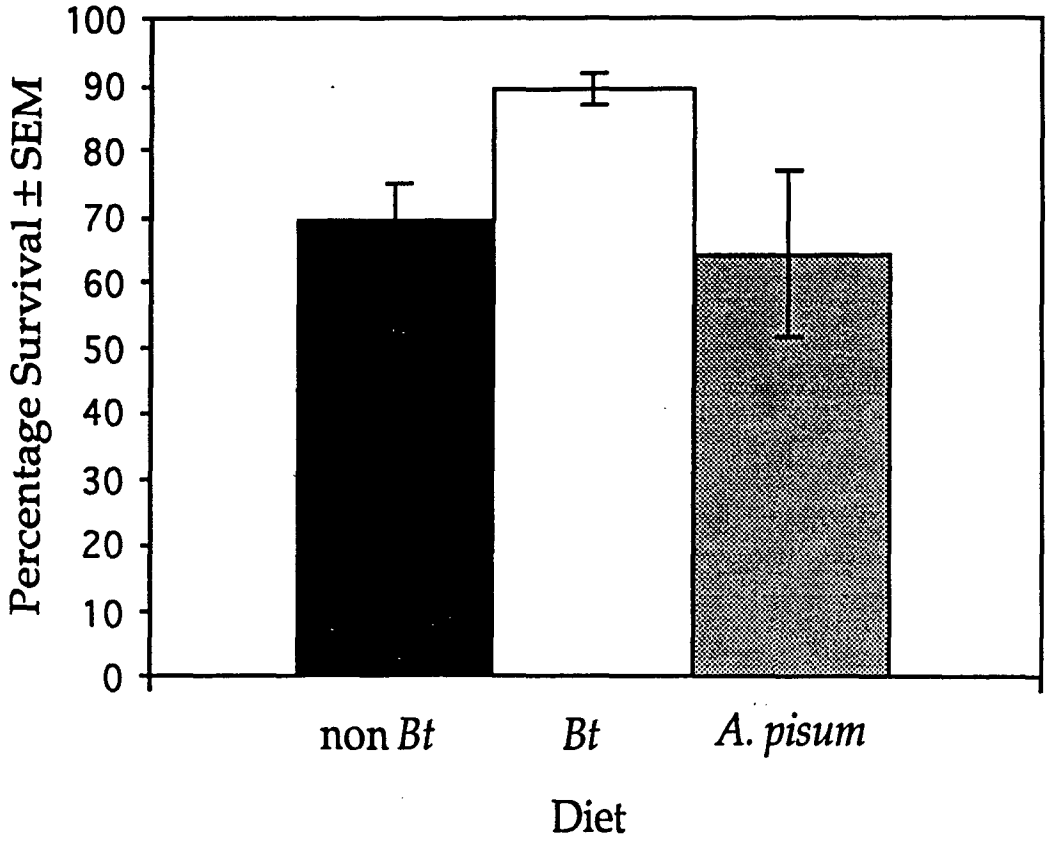

Figure 1. Mean percentage preimaginal survival ± SEM of *C. maculata* for three groups receiving one of three diets: non*Bt* pollen (non*Bt*), transgenic *Bt* pollen (*Bt*), or pea aphids, (*A. pisum*) at 26 ± 1°C, 16:8 L:D, ANOVA, ($F = 3.38$, $df = 2, 6$, $P = 0.10$).

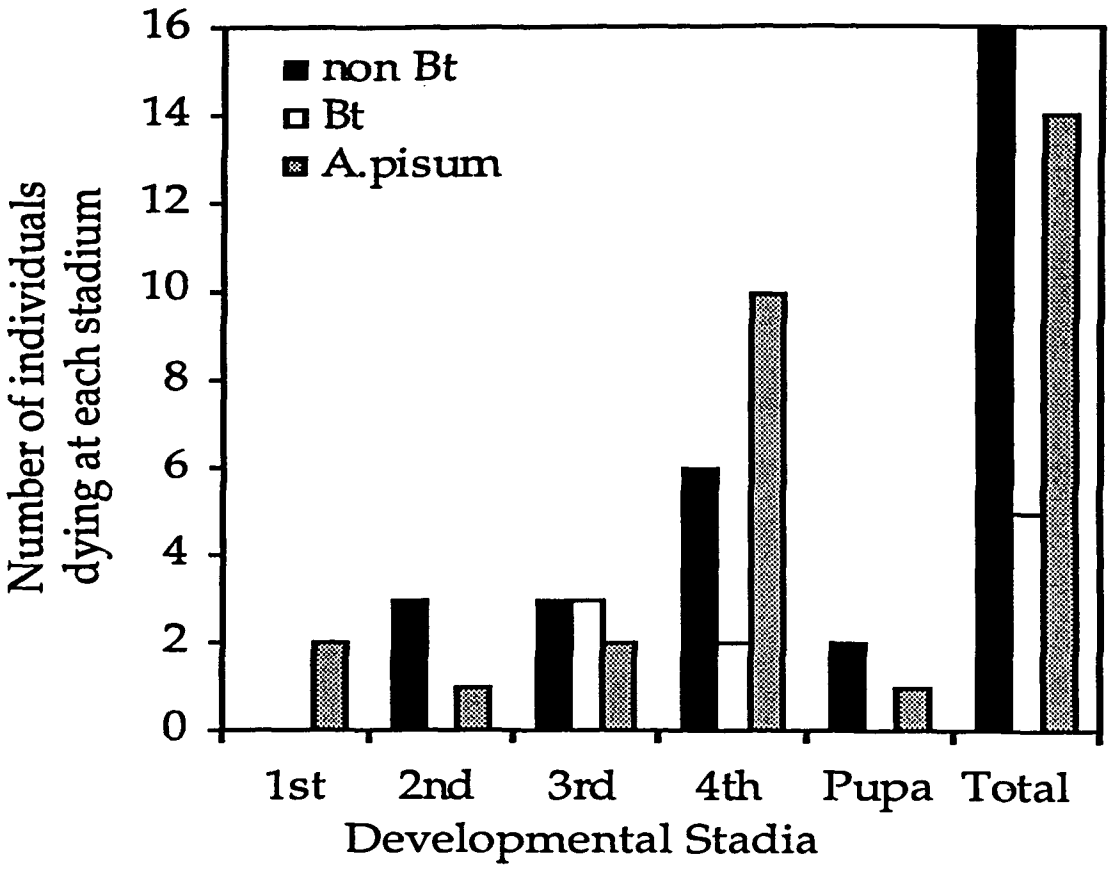

Figure 2. Stadium-specific mortality of *C. maculata* that failed to reach adult stage. Out of 45 individuals started per treatment, N = 16 died on *A. pisum;* N = 14 died on non *Bt* pollen; N = 5 died on *Bt* pollen.

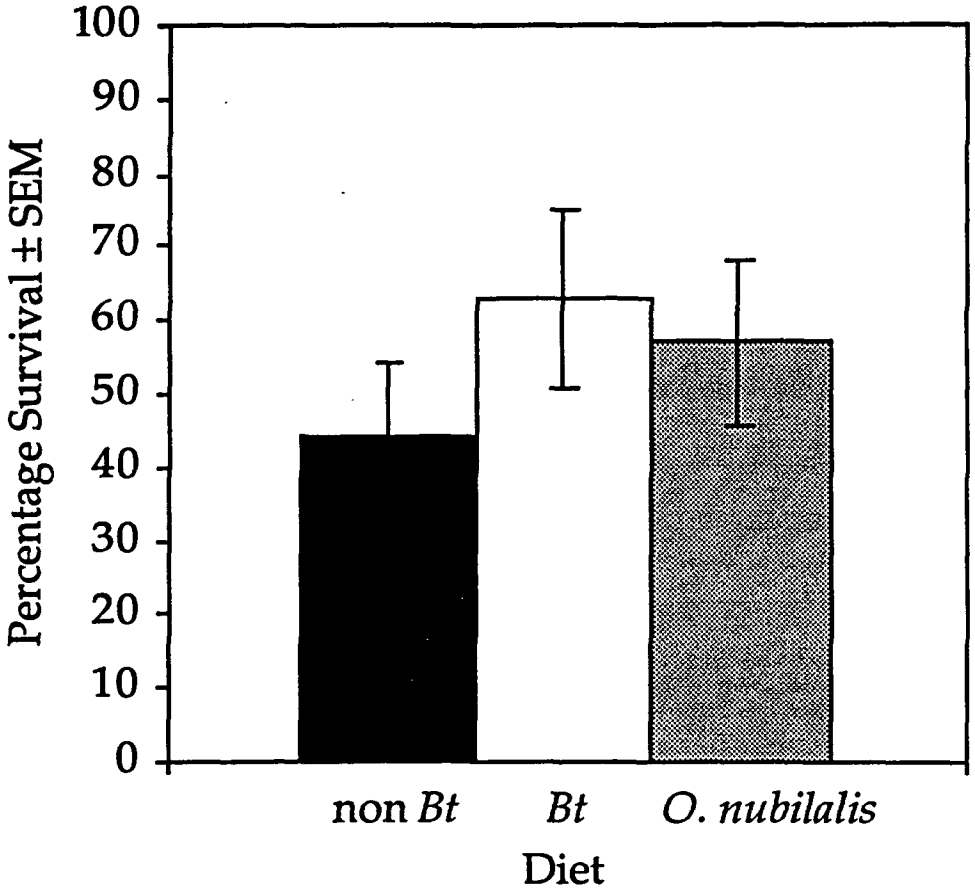

Figure 3. Mean percentage preimaginal survival ± SEM of *O. insidiosus* on three groups receiving one of three diets: non*Bt* pollen (non*Bt*), transgenic *Bt* pollen (*Bt*), or *O. nubilalis* egg masses at 26 ± 1°C, 16:8 L:D, ANOVA, ($F = 0.83$, $df = 2, 6$, $P = 0.48$).

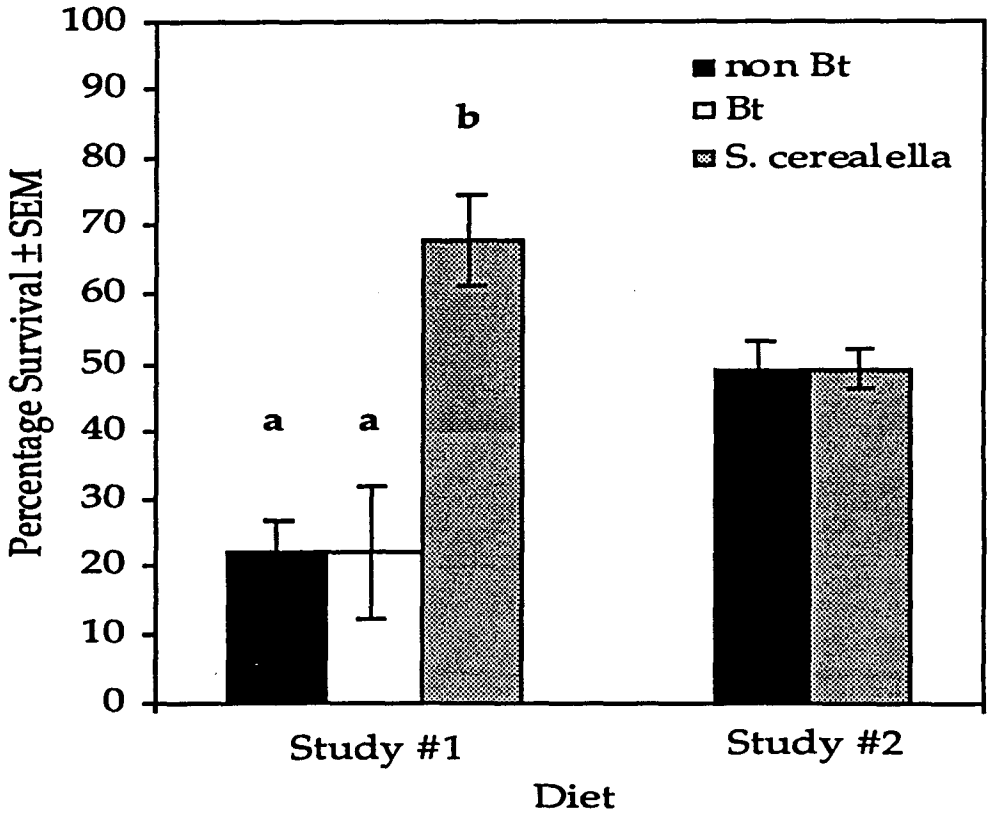

Figure 4. Mean percentage preimaginal survival of *C. carnea* for 2 studies maintained at 24 ± 1°C, 16:8 L:D. Significance is shown as different letters (Student *t*- test multiple comparisons) for study #1 among three groups receiving one of three diets: non*Bt* pollen, transgenic *Bt* pollen, or *S. cerealella* eggs, ANOVA, ($F = 9.5$, df 2, 6, $P = 0.01$). No significant difference was found in study #2 between two groups receiving non*Bt* pollen or transgenic *Bt* pollen, ANOVA, ($F < 0.01$, df = 1, 10, $P = 0.99$). No *S. cerealella* treatment was used in study #2.

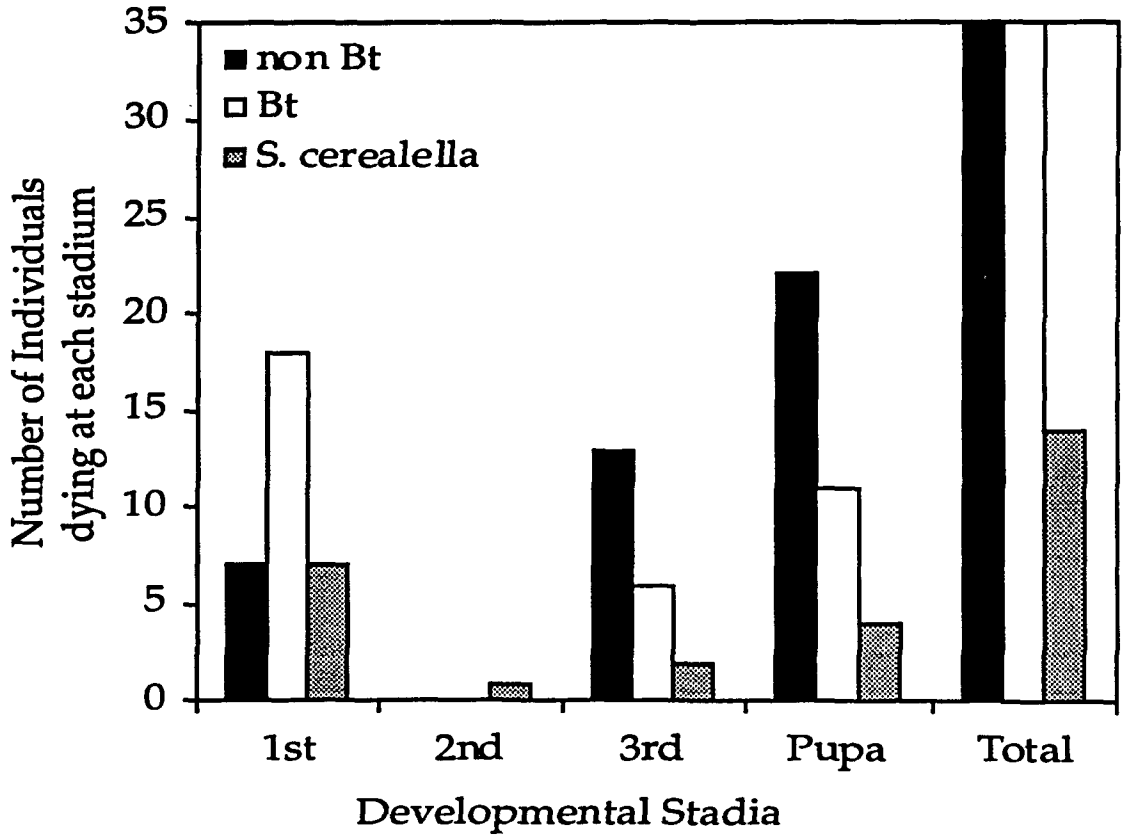

Figure 5. Stadium-specific mortality of C. *carnea* in study #1 that failed to reach adult stage. Out of 45 individuals started for each treatment, N = 14 died on *S. cerealella* eggs and N = 35 died on each pollen type.

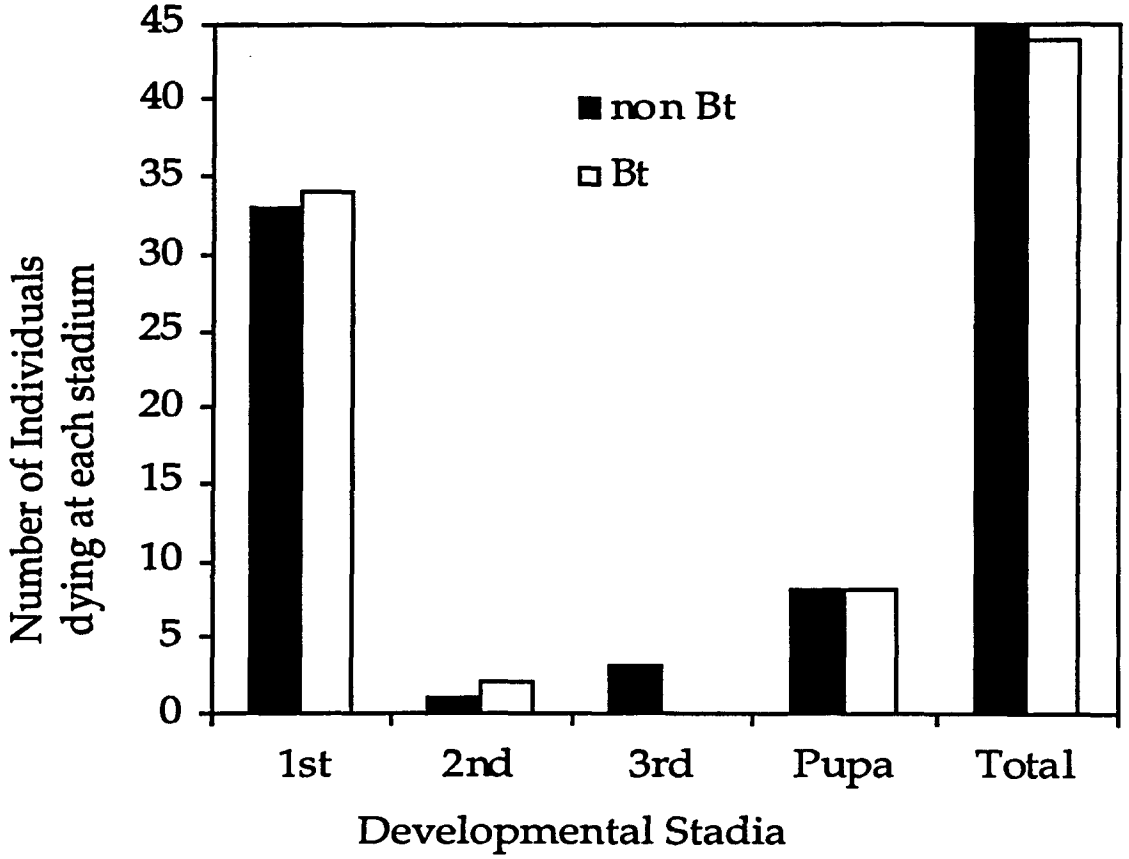

Figure 6. Stadium-specific mortality of *C. carnea* in study #2 that failed to reach adult stage. Out of 45 individuals started on each treatment, N = 45 died on non *Bt* pollen and N = 44 died on *Bt* pollen.

FIELD AND LABORATORY EVALUATIONS OF TRANSGENIC *BACILLUS THURINGIENSIS (BT)* CORN ON SECONDARY LEPIDOPTERAN PESTS

A paper to be submitted to *Journal of Economic Entomology*

C. D. Pilcher, M. E. Rice, J. J. Obrycki & L. C. Lewis

Abstract

In 1994 and 1995, field and laboratory studies evaluating the effects of transgenic *Bt* corn were conducted on the following noctuids: *Agrotis ipsilon* (Hufnagel), *Papaipema nebris* (Geunée), *Pseudaletia unipuncta* (Haworth), and *Helicoverpa zea* (Boddie). In 1994, *Bt* and non*Bt* leaf or silk tissues were collected from field grown plants and early instars were allowed to feed on the tissues for different periods of time in separate laboratory studies. In 1995, the Cry1Ab protein derived from *Bacillus thuringiensis* was extracted from field collected leaf tissue and topically applied to meridic diet at 20 ng/cm^2. Early instars from the four species were individually placed onto the diet which was treated with *Bt* protein extract, non*Bt* extract, or water. No *Bt* corn effects were observed on *A. ipsilon* or *P. nebris* indicated by no differences in larval survival, pupal weight, or days to adult emergence. However, lighter pupal weights (0.068 g), a delay in development (7.7 days), and trends for lower survival (11-25%) were observed by *P. unipuncta* reared on *Bt* extract. Low survival and significant delays in development (4 days) were observed for *H. zea*.

Transgenic *Bt* corn and non*Bt* corn was planted in a randomized complete block design with four replications in 1994 and 1995. Plants were artificially

infested (80 *Bt* and 80 non*Bt*) with early instars and later evaluated for damage (leaf feeding, stalk cutting, ear tip feeding). There were no differences between *Bt* and non*Bt* corn damage caused by *A. ipsilon*. *Papaipema nebris* caused significantly less leaf-feeding damage to *Bt* corn compared to non*Bt* corn in 1994. In addition, *P. unipuncta* and *H. zea* caused significantly less damage to leaf tissue both years. However, *H. zea* survived and caused damage to corn ears. There were fewer *Bt* corn ears damaged, but no difference in the number of live larvae per plant on *Bt* corn (1994 = 0.72, 1995 = 0.87) compared to non*Bt* corn (1994 = 0.81, 1995 = 0.79). Transgenic *Bt* corn does reduce feeding by *P. unipuncta* and *H. zea*.

Introduction

Susceptibility of a host and the specificity of a particular *Bt* crystal protein is dependent on two factors: 1) the larval gut affecting the solubilization and/or processing efficiency of the protein; and 2) the presence of specific toxin-binding sites (receptors) in the insect gut (Höfte & Whiteley 1989). These factors have led to the characterization of crystal proteins based on their structural similarities and insecticidal specificity. *Bt* genes that produce crystal proteins (*cry* genes) are classified into four groups, one being a lepidopteran-specific protein (130 kDa) derived from a *cry1* gene. Lepidopteran-specific Cry1 proteins are initially protoxins, which solubilize within the insect gut (depending on the proper pH) to produce the active toxin responsible for causing midgut paralysis (Höfte &

Whitely 1989, Gill et al. 1992). Recognizing the mode of action for *Bt* complements the understanding of why various insects have different levels of susceptibility to different crystal proteins.

Many studies report the susceptibility of insects to different isolates of *Bt* protein under laboratory conditions (Burges 1981, Jaquet et al. 1987, Höfte et al. 1988, MacIntosh et al. 1990, Stone & Sims 1993, Ebora et al. 1994, Sims 1995), and in field conditions (Miller 1990, Ali & Young 1993, Bartels & Hutchison 1995, Johnson et al. 1995). Insect susceptibility and efficacy was evaluated in laboratory studies using purified *Bt* proteins in insect bioassays (Jaquet et al. 1987, Höfte et al. 1988, MacIntosh et al. 1990, Stone & Sims 1993, Sims 1995). In addition, field studies have evaluated *Bt* microbial sprays to determine susceptibility of target and nontarget insects (Miller 1990, Ali & Young 1993, Bartels & Hutchison 1995, Johnson et al. 1995). Recent advances in biotechnology have allowed for the development of plants that express *Bt* protein (Warren et al. 1992, Benedict et al. 1993, Fujimoto et al. 1993, Koziel et al. 1993, Perlak et al. 1993), but it is impossible to translate past research with *Bt* sprays to the expected performance of transgenic plants.

Field corn, *Zea mays* (L.), has been genetically engineered to express the Cry1Ab protein derived from *Bt* (Koziel et al. 1993) with the native gene synthetically modified to produce adequate expression levels of *Bt*. The truncated protein produces the same insecticidal toxin in the insect midgut even though this protein produced by the plant is only 65% homologous to the

original and the delivery method (corn plants producing Cry1Ab protein) to the pest is radically different from previous application strategies (Koziel et al. 1993, Bryant 1994). Corn plants are capable of continual expression of the protein through the growing season allowing constant protection against the European corn borer, *Ostrinia nubilalis* (Hübner).

Few studies have evaluated the effects of *Bt* against secondary or nontarget lepidopteran species. It has been reported that *Bt kurstaki* sprays are toxic to some nontarget lepidopterans (Miller 1990, Johnson et al. 1995). In addition, MacIntosh et al. (1990) and Sims (1995) report the susceptibility and efficacy of several agronomically important insects to purified *Bt* proteins (Cry1Ab, Cry1Ac, Cry1AcT (trypsin resistant core) and Cry3A) and found varying levels of activity against many insects including *Agrotis ipsilon* (Hufnagel), *Helicoverpa zea* (Boddie), *Heliothis virescens* (Fabr.) and *O. nubilalis*. Only one study, however, has reported the effects of transgenic *Bt* plants (potato) on secondary lepidopteran insects (Ebora et al. 1994); *Manduca sexta* (L.) was the target pest, but the transgenic *Bt* potato showed significant effects on secondary pests *Phthorimea operculella* (Zeller) and *O. nubilalis*. *Ostrinia nubilalis* is the target pest for transgenic *Bt* corn, but there are several other economically important corn pests occasionally attacking the crop: black cutworm (*A. ipsilon*), stalk borer (*Papaipema nebris* (Geunée)), armyworm (*Pseudaletia unipuncta* (Haworth)), and corn earworm (*H. zea*). The effects of transgenic *Bt* corn on these secondary

corn pests have not previously been reported, but could be relevant to an integrated pest management program in corn.

The objectives of this study were to: 1) determine the susceptibility of *A. ipsilon, P. nebris, P. unipuncta,* and *H. zea* to transgenic *Bt* corn in the laboratory; and 2) compare the field efficacy of transgenic *Bt* corn on these corn pests to that of non*Bt* corn.

Materials and Methods

Field Studies

Studies in 1994 and 1995 measured the effects of transgenic *Bt* corn on secondary lepidopteran pests. Field plots were located at the Insectary, Iowa State University, Ames, IA. In 1994, transgenic *Bt* and non*Bt* corn (CIBA hybrid cross CG00554 x CG00526) was hand planted in four row plots, 3 x 7.6 m (10 x 25 ft) in a randomized complete block design with four replications for each insect species. Plots were planted on 2 May, 1994. In 1995, the same methods including the plot plan, planting technique, and location were used, however, a packaging problem resulted in different hybrids being planted for transgenic *Bt* plots (CIBA hybrid 4403E) and non*Bt* plots (CIBA hybrid 4490). However, both transgenic *Bt* hybrids each year expressed the same Cry1Ab protein and originated from the same transformation event - 176 (Koziel et al. 1993). These plots were planted on 20 May, 1995. The two middle rows of each plot were used for testing each year with the two outside rows acting as buffer rows. Each year rows were planted with a

0.76 m (30 in) spacing at a rate of 64,467 seed/ha, 26,100 seed/A (9 in. seed spacing).

Field study results were based on damage ratings given to each of 20 corn plants within each plot (80 *Bt* and 80 non*Bt* plants evaluated). Rating scales varied among species (description described below for each species). Means were analyzed using ANOVA (corn type x replication) at P = 0.05 (SAS Institute Inc. 1995, JMP 3.1, Cary, NC). Studies evaluating *H. zea* damage (categorical data) to ears were analyzed using a Chi-square analysis (SAS Institute, Inc. 1995). Years were analyzed separately because a different hybrid was used each year. Leaf tissue from both years was collected and sent to Ciba Seeds (Research Triangle Park, NC) to confirm *Bt* protein presence. The levels in *Bt* corn plants were > 1,800 ng/g dry weight which is sufficient to provide 70% mortality of first instar *O. nubilalis* in a 48 hr bioassay (Laura Privalle, Ciba Seeds, personal communication).

Laboratory Studies

Laboratory feeding studies were conducted for each species tested in the field. *Agrotis ipsilon* and *P. unipuncta* larvae were obtained from the USDA-ARS Corn Research Laboratory in Ames, IA. *Helicoverpa zea* larvae were obtained from the Iowa State University Plant Introduction Station, Ames, IA. *Papaipema nebris* larvae were collected from smooth brome grass, *Bromus inermis* Leyssera, near Ames, IA. All corn plant material was collected from the field plots. In 1994, the larvae were initially given either transgenic *Bt* or non*Bt*

leaf or silk tissue as a food source for different periods of time depending on the study (description below for each species). Larvae were fed approximately 2 cm^2 leaf tissue or 20 5 cm silk strands (*H. zea*) in 7-dram vials. Larvae were later transferred to a meridic diet prepared for black cutworm, but it works equally well for other noctuid species tested (Hendrix et al. 1991). Larvae were maintained at 26 ± 1°C , 16:8 L:D. Survival, pupal weights, and developmental times were recorded.

In 1995, corn leaves were collected from the field, lyophilized (VirTis, Gardiner, N.Y.), and then mixed with a buffer solution (50 mM $NaHCO_3$, 0.1 M NaCl) to extract the Cry1Ab protein from the tissue (Laura Privalle, Ciba Seeds, personal communication). The solution was centrifuged for 10 minutes at 16,000 revolutions per minute (Damon centrifuge, IEC, B-20A). Insect rearing trays (BioServ 9 x 11mm, 30 cells) were filled half-way with the noctuid meridic diet. Diet cells were each topically treated with 75 µl of extract (removed from centrifuge tubes), spread evenly across the surface (0.81 cm^2) and allowed to dry for 30 minutes. The remaining extract was then frozen at - 80°C and sent overnight mail to Ciba Seeds Agricultural Biotechnology Research Station for measurement of *Bt* protein concentrations. Concentration levels exceeding 220 ng *Bt*/ml were observed in all samples tested, which are adequate to kill early-instar *O. nubilalis*. The dose applied to the diet in 1995 studies was in excess of 20 ng Cry1Ab/cm^2. Larvae of each species (*A. ipsilon, P. unipuncta,* and *H. zea*) were placed one per cell and sealed with a heated plastic tray sealer (Oliver

Products, Co., Grand Rapids, MI). Larvae received either *Bt* corn extract, non*Bt* corn extract or water as treatments. The water treatment was added to determine if the corn extract was having any detrimental effects on larvae. The 1995 methods were used to decrease the handling of larvae that may have caused unnecessary mortality in 1994 studies. In addition, the larvae were exposed to equal concentrations of the Cry1Ab protein for the same periods of time. Larvae were maintained at 24 ± 1°C, 16:8 L:D. Developmental times, pupal weights, and survival were recorded. Data from all laboratory studies are presented. *Ostrinia nubilalis* larvae were used as a control in 1995.

Developmental data, survival, and pupal weights were analyzed using Student's *t* -test in 1994 (2 treatments) and ANOVA in 1995 (3 treatments) at P = 0.05. Larval survival was analyzed using the means from four groups of ten individuals in each study. Proportional data was transformed to arcsin of the square root of these proportions before analysis. Nontransformed data are presented.

Agrotis ipsilon

Field studies with *A. ipsilon* were initiated on 20 May, 1994, and 6 June, 1995, when the corn was at V2-stage (Ritchie et al. 1993) development. Five first instars were placed into the whorl of each plant with a camel-hair brush (80 *Bt* and 80 non*Bt* plants). Coffee-tin cans (#10) were placed around each plant to prevent larval migration. Each leaf of every plant was evaluated three days later for damage using the following scale: 1 = no leaf feeding, 2 = light skeletonizing

(leaf feeding < 1/2 of leaf), 3 = heavy skeletonizing (leaf feeding > 1/2 of leaf), 4 = leaf cut or dead. For analysis, the average leaf rating per plant was calculated and recorded on a per plant basis.

A second study was conducted each year to measure *A. ipsilon* cutting from late-stage larvae. Separate plants were used in the same plots and two fourth instars were placed on each plant on 23 May, 1994, and 2 June, 1995. Corn was in the two or three-leaf stage of development. Plants were analyzed twice following the infestation and given a damage rating: 1 = no feeding or cutting, 2 = leaf feeding and skeletonizing, 3 = one leaf cut and feeding, 4 = two or more leaves cut, 5 = stalk cut above ground, 6 = stalk cut below ground. This scale was slightly adjusted from Johnson & Lewis (1982). The damage ratings from the final evaluation each year was subject to analyses.

Laboratory studies were conducted simultaneously with the field studies. In 1994, first and second instars were placed on emerging whorl leaves of 40 *Bt* and 40 non*Bt* plants in the field. Larvae contained on the leaf using a tape shape (Converters, Inc., Huntingdon Valley, PA) clipped to the leaf with a curl clip (Styling Essentials, E. Rutherford, NJ) were allowed to feed for 48 hr. Mortality was recorded after 48 hr. and live larvae were transferred to a meridic diet as described earlier. Larvae were monitored daily for mortality. The number of days until pupation and pupal weights were recorded and then larvae were separated by sex. In 1995, a different method was used because of escapes and excessive handling of larvae in 1994. Whorl-leaf tissue was collected from 10-leaf

stage *Bt* (CIBA hybrid 4403E) and non*Bt* (CIBA hybrid 4490) corn for the insect bioassays. The leaf tissue was lyophilized and then frozen at -20°C until protein extraction (described above). Larvae were examined for early mortality seven days following exposure to the treatments. Developmental data, survival and pupal weights were analyzed and then sexes were separated and analyzed. A second 1995 study was initiated using *Bt* and non*Bt* leaf tissue from the same hybrid (CIBA 4401). These plants were grown at the same location later in the summer. Leaf tissue was collected from three-leaf stage corn. The same procedures were used and identical variables were examined, however larvae were checked four (instead of seven) days following exposure for any toxic effects.

Papaipema nebris

Field studies began on 31 May, 1994. Whorls of 160 plants (V4-stage corn) (80 *Bt*, 80 non*Bt*) were infested with one second or third instar per plant with a camel-hair brush. Five-gallon plastic buckets (bottoms removed) were placed around plants to help prevent larval migration. The plots were evaluated biweekly to monitor injury progress of plants using the following scale (Davis & Pedigo 1990): 1) plant uninfested or minor leaf feeding present; 2) plant tunneled, very little leaf feeding, and growing point is not injured; 3) heavy leaf feeding, growing point not injured; 4) dead heart, growing point not injured; 5) dead heart and plant tillers and; 6) plant killed. Only final ratings are reported. No studies were completed in 1995.

Laboratory studies were conducted simultaneously with field studies. In 1994, 80 larvae were collected from smooth brome grass and placed on the whorl leaves of seedling plants (40 *Bt*, 40 non*Bt*) in the field. They were contained on the leaves using tape shapes and curl clips for 72 hours and then transferred to meridic diet. Larvae were monitored daily for mortality. Developmental time, pupal weights and survival were recorded and adults were separated by sex for analysis. In 1995, a different method was used because escape and excessive handling of the larvae occurred in 1994. *Papaipema nebris* eggs began hatching in the field around 9 May 1995. Transgenic *Bt* and non*Bt* leaf tissue (CIBA hybrid cross CG00554 x CG00526) collected and frozen in 1994 was lyophilized and then incorporated into a meridic diet. Approximately 1500 ng *Bt* protein was incorporated into each cell for those larvae that received the *Bt* treatment. Ninety-six larvae received one of three treatments: *Bt* leaf protein diet, non*Bt* leaf diet, or meridic diet. Larvae were checked three days later for mortality and then monitored for developmental events, i.e. pupation, eclosion, and mortality. Developmental time, pupal weights, and survival were recorded and adults were separated by sex.

Pseudaletia unipuncta

Field studies were initiated on 15 June 1994 and 19 June 1995. Plants (80 *Bt*, 80 non*Bt*) were artificially infested in 1994 with 15-20 first instars. In 1995, plants received 20-25 first instars on three dates, 19, 27, and 28 June. No larvae survived in the field either year so the amount of feeding was measured by

counting the number of feeding scars and dividing them into two categories, scars < 2 cm and scars > 2 cm.

Three laboratory studies were completed in 1994 and two in 1995. In the first 1994 study, single first instars were placed on whorl leaves (40 *Bt*, 40 non*Bt*) and allowed to feed for 48 hours while contained by tape shapes and curl clips. Mortality was recorded and larvae were transferred to a meridic diet and monitored daily. In the second study, second instars were placed into 7-dram vials and allowed to feed for 24 hours on fresh whorl tissue (2 cm^2) collected from the field. They were then transferred to meridic diet and monitored for development. In the third 1994 study, first instars were allowed to feed for 72 hours on whorl leaf tissue in 7-dram vials, and then transferred to meridic diet. Developmental time, pupal weights, and survival were recorded and then adults were separated by sex. In 1995, the same procedures were used as described above under *A. ipsilon*. In the first study, larvae were checked seven days following initial exposure for survival. In the second study, larvae were checked four days following exposure. In the second study, days to eclosion are not reported because a mite infestation killed all the pupae.

Helicoverpa zea

Field studies were initiated on 14 July, 1994, and 29 July, 1995 when ear silks (80 *Bt*, 80 non*Bt*) were infested with five first instars. Ears were evaluated two weeks later and received ratings of 1 for no damage or 2 for damage. Live larvae per ear also were counted. Some ears were damaged by raccoons, *Procyon*

lotor, and discarded from sampling. In 1995, V6 plants received 20-25 first instars in the whorl. The number of leaf-feeding scars and larvae located in the whorls were counted. No leaf-feeding studies were completed in 1994 because rain following infestations drowned the larvae.

In two 1994 laboratory experiments, first-instars were contained in 7-dram vials and given either whorl leaf or silk tissue (40 *Bt*, 40 non*Bt*) and allowed to feed for 72 hours. The larvae were then transferred to a meridic diet. In 1995, procedures described for *A. ipsilon* and *P. unipuncta* were used. First instars were exposed to the treated meridic diet in two experiments. In the first study, larvae were checked for mortality five days following exposure and six days following exposure in the second study. Developmental time, pupal weights, and survival were recorded and then adults were separated by sex both years.

Results

Agrotis ipsilon

In the laboratory studies, survival was the only statistically significant ($P < 0.05$) difference observed in 1994 (Table 1). Larvae reared on *Bt* leaf tissue had higher survival (18%) than those reared on non*Bt* leaf tissue. In 1995 study #1, pupal weights of individuals reared on *Bt* protein extract averaged 0.04 g heavier than larvae on non*Bt* extract ($P < 0.05$). All other developmental variables showed no differences between *Bt* and non*Bt* corn effects on *A. ipsilon*. There are trends for lower survival of larvae exposed to *Bt* in the 1995 studies (13 -

20%), but no significant differences were observed. In the field studies, means were not significantly different between *Bt* (1994 = 2.08, 1995 = 2.08) and non*Bt* (1994 = 2.10, 1995 = 2.15) leaf damage ratings (ANOVA, 1994: F = 0.07, P = 0.79, df = 1, 155; 1995: F = 1.23, P = 0.27, df = 1, 155; Fig. 1). There were also no differences between *Bt* (1994 = 3.19, 1995 = 5.84) and non*Bt* (1994 = 3.38, 1995 = 5.85) in plant cutting ratings (ANOVA, 1994: F = 1.01, P = 0.32, df = 1, 155; 1995: F = 0.02, P = 0.90, df = 1, 155; Fig. 2).

Papaipema nebris

There were no differences observed in developmental responses of larvae in the *Bt* or non*Bt* treatments (Table 2). In 1994, a slightly higher percentage (8%) of larvae survived on *Bt* leaf tissue compared to non*Bt* leaf tissue. However, in 1995 there were trends for lower survival for *Bt* treated larvae (44 ± 9.6%) than was observed from non*Bt* treated larvae (61 ± 7.1%), although there was no significant difference (Table 2). Field studies showed slightly different results. There was significantly less damage to *Bt* corn compared to non*Bt* corn in 1994 (ANOVA, F = 10.99, P = 0.0011, df = 1, 155; Fig. 3).

Pseudaletia inipuncta

In 1994, there were statistically significant differences in developmental responses between treatments suggesting an effect on larvae fed *Bt* leaf tissue (Table 3). In study #2, male larvae exposed to *Bt* leaf tissue were significantly lighter in pupal weight by 0.035 g than those exposed to non*Bt* leaf tissue. This is in contrast to observations in study #3 where females exposed to *Bt* leaf tissue

were significantly heavier by 0.039 g than those receiving non*Bt* leaf tissue. There were trends in all three 1994 studies for lower survival (11-23%) of larvae reared on *Bt* leaf tissue compared to non*Bt* leaf tissue, but significant differences were not detected.

In 1995 study #1, larvae fed the *Bt* diet took approximately 5 days longer to complete pupation. Female pupae on *Bt* diet weighed 0.077 g less than those reared on non*Bt* diet and males reared on *Bt* diet weighed 0.060 g less than those on non*Bt* diet. In addition, these same larvae took longer (females = 5.8 days; males = 9.2 days) to reach the adult stage compared to non*Bt* treatments. Similar results were observed in 1995 study #2 where females and males reared on *Bt* diet took approximately 4 days longer to pupate. Again, there were trends for lighter pupal weights (> 0.026 g) from individuals reared on *Bt* diet in both sexes, but these were not statistically different from those reared on non*Bt* diet. In study #2, individuals did not complete development.

The damage to *Bt* corn in the field (feeding scars < 2 cm) was significantly less than the damage to non*Bt* corn by 36% in 1994 and by 57% in 1995 (ANOVA, 1994: $F = 13.5$, $P = 0.0003$, $df = 1, 155$; 1995: $F = 304.2$, $P < 0.0001$, $df = 1, 155$; Fig. 4). In addition, larger feeding scars (> 2 cm) were also counted and again, less damage occurred on *Bt* corn compared to non*Bt* corn by 91% in 1994 and 93% in 1995 (ANOVA, 1994: $F = 9.64$, $P = 0.002$, $df = 1, 155$; 1995: $F = 8.7$, $P = 0.004$, $df = 1, 155$; Fig. 5). Because larvae did not survive in the field for very long, the number

of feeding scars from early stage larvae were used to indicate the effects *Bt* corn would have on *P. unipuncta.*

Helicoverpa zea

In 1994, there were trends in laboratory studies indicating detrimental effects of *Bt* corn on *H. zea.* There were no survivors of larvae fed *Bt* leaf tissue (Table 4). In addition, fewer individuals survived of those larvae fed *Bt* corn silks compared to those fed non*Bt* silks. However, there were no significant differences in these responses in 1994. In 1995, differences in deveopmental responses were observed from *H. zea* that were also observed by *P. unipuncta.* However, *H. zea* showed no differences in survival or pupal weights (Table 4). Larvae exposed to *Bt* protein extract required approximately 3 days longer to reach the adult stage compared to those larvae exposed to non*Bt* extract. However, larvae exposed to *Bt* protein extract had slightly higher survival (15%) than those exposed to non*Bt* extract in 1995 study #1 ($P < 0.05$).

Larvae placed onto ear tips damaged fewer *Bt* ear tips than non*Bt* ear tips in 1994 and 1995 (Chi-square, 1994: $x^2 = 8.76$, $P = 0.0039$; 1995: $x^2 = 22.06$, $P < 0.0001$; Fig. 6). Larvae caused damage to 85% of the non*Bt* ears and only 57% of the *Bt* ears. However, no differences were observed either year in the number of larvae found on *Bt* ears (1994 = 0.72, 1995 = 0.87) compared to non*Bt* ears (1994 = 0.81, 1995 = 0.79) (ANOVA, 1994: $F = 0.70$, $P = 0.41$, $df = 1, 148$; 1995: $F = 0.44$, $P = 0.51$, $df = 1, 145$; Fig. 7). In the leaf-feeding study, there were significantly fewer (62%) feeding scars on *Bt* corn plants from first instars compared to the number of scars

on non*Bt* plants (ANOVA, 1995: $F = 123.3$, $P < 0.0001$, $df = 1, 153$; Fig. 8). In addition, there were no larvae observed feeding on *Bt* plants compared to an average of one larva per five plants feeding on non*Bt* plants (ANOVA, 1995: $F = 19.9$, $P < 0.0001$, $df = 1, 153$; Fig. 9).

Bioassays evaluating *O. nubilalis* response to *Bt* protein extract were conducted simultaneously with the studies on the secondary lepidopterans in 1995. In 1995 study #1, only one larva (2.5%) survived on *Bt* protein extract compared to 87.5% survival on non*Bt* extract. Similar results were observed in 1995 study #2 when 7.5% of the larvae reared on *Bt* protein extract survived compared to 95% survival on non*Bt* extract.

Discussion

There were no *Bt* protein effects in either the laboratory or field studies on *A. ipsilon*. This corroborates the findings of MacIntosh et al. (1990) where purified Cry1Ab protein showed very little activity against *A. ipsilon* ($LC_{50} > 80$ µg/ml). Our results from the laboratory were expected, but little was known about the potential response of *A. ipsilon* to *Bt* corn in the field. This insect is known for cutting damage to small plants and can cause the highest yield losses to corn when fourth through sixth instars feed on plants at the coleoptile to four-leaf stage (Showers et al. 1983, Whitford et al. 1989). In 1995, both *Bt* and non*Bt* plants received similar levels of damage to the point where > 80% of the plants were killed. We observed that *A. ipsilon* is not affected by the *Bt* protein

(Cry1Ab) expression in leaves even though high expression levels of the protein have been observed in the leaves (Koziel et al. 1993). *Agrotis ipsilon* has shown susceptibility to *B.t. thuringiensis* (Burges 1981), so there is potential for using a *Bt* crystal protein to control this pest.

For *P. nebris,* there were no differences in developmental time, survival, or pupal weights in either *Bt* or non*Bt* treated larval groups. However, differences were observed in the field study. In 1994, there was less damage to *Bt* corn, however we were unable to collect data a second year to support these results. *Papaipema nebris* larvae may show a preference for non*Bt* corn over *Bt* corn. Farmers may benefit from the effect *Bt* corn has on *P. nebris.* Yield loss per plant from *P. nebris* is comparable to losses observed from *A. ipsilon* and *O. nubilalis* (Bailey & Pedigo 1986, Davis & Pedigo 1991). Once the larvae get too large for smaller-stemmed hosts like smooth brome, *B. inermis,* and orchard grass, *Dactylis glomerata* L., larvae of *P. nebris* reportedly migrate from neighboring grassy areas into neighboring corn plants early during corn development (Decker 1931, Lasack & Pedigo 1986). Larvae feed on leaf axils and within the whorl before boring into corn stalks (Lasack & Pedigo 1986).

Davis and Pedigo (1990) proposed a management strategy for applying insecticide during larval movement and whorl feeding to reduce damage to corn rows adjacent to terraces and roadsides. In our study, larvae were enclosed on corn and not given the opportunity for interplant movement. However, if larvae are able to detect *Bt* protein in leaves of *Bt* corn plants, they may continue

to migrate from one corn plant to another. These larvae would potentially be more vulnerable to predation and other mortality factors because they would be exposed for a longer period of time.

Our studies show that *Bt* corn significantly affects *P. unipuncta.* In 1994, larvae of different ages were exposed to *Bt* and non*Bt* leaf tissues for three different time periods. We observed no differences in developmental responses between the corn types among the three different time periods tested. Trends in all three studies show lower survival of larvae that fed on *Bt* leaf tissue. These results were again observed in 1995, however delayed development and lighter pupal weights also occurred in larvae exposed to *Bt* treated diet. The differences between 1994 and 1995 was that larvae were exposed to *Bt* in a different form (corn tissue compared to diet) and for a longer period of time in 1995. These results suggest that approximately 20% of the larvae are susceptible at the dosage used (20 ng Cry1Ab/cm^2) and observed differences probably result from a physiological response. The larvae may have completely stopped feeding for a period of time or adjusted their feeding pattern to allow enough time to recover from sublethal doses of *Bt* within the midgut. Those larvae that received a lethal dose died early as shown by the 4 and 7 day survival checks in the 1995 studies. Those larvae that received a sublethal dose survived, but were significantly delayed in development and produced lighter pupae. Bioassays using the same *Bt* doses killed 97.5% of the *O. nubilalis* first instars during the same 4 to 7 day

time period. *Pseudaletia unipuncta* first instars are not as susceptible to Cry1Ab protein as *O. nubilalis* first instars.

Even though there was no larval establishment in our field studies because of weather related problems, initial feeding by laboratory-reared first instar *P. unipuncta* placed on corn plants resulted in less damage to *Bt* corn. Less larval feeding occurred because larvae either moved off of *Bt* corn or were killed. This may benefit growers economically because *P. unipuncta* has been shown to be an occasional serious pest of corn in the north central Corn Belt (Rice 1993). These insects are significant leaf-defoliators and tend to cause damage to V7-V8 stage corn (Mulder & Showers 1986). In addition, fourth instars were shown to do the most damage and cause the greatest yield losses (Mulder & Showers 1986). Although our studies were conducted with first instars, field and laboratory results suggest that *Bt* corn could impact *P. unipuncta* during all stages of development if we consider their potential ability to detect *Bt* within the tissue. Further studies are needed to evaluate effects of *Bt* corn on larger instar *P. unipuncta* because the likelihood of first instars attacking corn is smaller compared to larger instars.

Transgenic *Bt* (Cry1Ab) corn significantly affects *H. zea*. We observed trends in 1994 for 20% lower survival of larvae reared on *Bt* leaf and silk tissues and delays in development by individuals reared on *Bt* protein extract in 1995. In addition, percent survival in 1995 was 98 and 88% in bioassay studies #1 and #2, respectively, compared to 2.5 and 7.5% observed survival in the *O. nubilalis*

control groups. These results correspond with those observed by MacIntosh et al. (1990) where *H. zea* were 10 - fold less susceptible to Cry1Ab compared to *O. nubilalis*. Also, Sims (1995) observed higher survival (34.7%) by *H. zea* compared to *O. nubilalis* (9.7%) when rearing individuals on a Cry1Ac treated diet. In addition, susceptibility of *H. zea* to *Bt* has shown to range greatly among different populations (>13 fold) (Stone & Sims 1993). The relationship between susceptibility to *Bt* in the laboratory and efficacy in the field remains to be established (Stone & Sims 1993).

We conducted field studies and observed similar results to what was observed in the laboratory. *Helicoverpa zea* require a higher dose of Cry1Ab protein to be affected to the same extent as *O. nubilalis*. A significant delay occurred in development, but there was no difference in survival between individuals reared on *Bt* and non*Bt* diets in 1995 laboratory bioassays. In the field, there were fewer *Bt* ears that were damaged, but no differences in the number of larvae between corn types. There were more larvae/damaged ear on the *Bt* corn compared to non*Bt* corn. In addition, larvae on *Bt* corn were one or two instars delayed in development compared to those on non*Bt* corn. Due to the delay, larvae may have been unable to compete effectively with other surviving larvae on the same ear, thus the higher number of survivors. On the undamaged ears, larvae either died from *Bt*, predators or left the plants. Corn ears (CIBA 3906 and 4403) containing lower expression levels of *Bt* (Koziel et al.

1993) do affect *H. zea* larvae, but not to the extent that complete protection is observed.

Conversely to what was observed on corn ears, larvae placed in leaf whorls reacted quite differently. There were fewer feeding scars on *Bt* plants and there was no larval survival compared to one live larva per 5 plants on the non*Bt* corn. The differences in the number of larvae between the two parts of the plant (leaf and ear) probably relate to the levels of *Bt* protein expression in these different tissues. The protection observed on the ears can be compared to observations from Bartels & Hutchison (1995). They reported low efficacy (34% control) of aerially applied *Bt* on *H. zea* unless the *Bt* was combined with a synthetic-insecticide. Control of *H. zea* on ear tips of *Bt* corn (CIBA 3906 and 4403) is comparable to that observed from the aerial *Bt* application.

The potential to obtain better control of *A. ipsilon, P. nebris, P. unipuncta,* and *H. zea* may be possible in the near future. Transgenic *Bt* corn that is currently being marketed will impact *P. unipuncta* and *H. zea,* but the level of control is minimal compared to that observed for *O. nubilalis.* These results are limited to one transgenic *Bt* event and the differences between hybrids in their expression and concentrations of *Bt* in different tissues are expected. Control of secondary lepidopterans may vary dependent upon which hybrids are planted.

Acknowledgments

We thank undergraduate workers J. Ostrem, C. Grutzmacher, T. Cortilet, and E. Butin for field and laboratory assistance. We thank Dr. Paul Hinz, Department of Statistics, Iowa State University, for statistical advice. We thank Jean Dyer, USDA-ARS Corn Research Laboratory, and Sharon McClurg, Iowa State University Plant Introduction facility for supplying larvae. This research was supported in part by Ciba Seeds Agricultural Biotechnology Group, Leopold Center for Sustainable Agriculture grant and Dupont Chemical in the form of a Student Scholar award. C.D.P. was supported by a research/extension assistantship from the Department of Entomology, Iowa State University. Journal Paper No. J-xxxxx of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 2755.

References Cited

Ali, A. & S. Y. Young. 1993. *Bacillus thuringiensis* var. *kurstaki* activity against larvae of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) on cotton. J. Econ. Entomol. 86: 1064-1068.

Bailey, W. C. & L. P. Pedigo. 1986. Damage and yield loss induced by stalk borer (Lepidoptera: Noctuidae) in field corn. J. Econ. Entomol. 79: 233-237.

Bartels, D. W. & W. D. Hutchison. 1995. On-farm efficacy of aerially applied *Bacillus thuringiensis* for European corn borer (Lepidoptera: Pyralidae) and corn earworm (Lepidoptera: Noctuidae) control in sweet corn. J. Econ. Entomol. 88: 380-386.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone & S. R. Sims. 1993. Impact of ∂-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Bryant, J. E. 1994. Application strategies for *Bacillus thuringiensis.* Agriculture, Ecosystems, and Environment. 49: 65-75.

Burges. 1981. Microbial control of pests and plant diseases 1970-1980. Academic Press, New York, NY.

Davis, P. M. & L. P. Pedigo. 1990. Evaluation of two management strategies for stalk borer, *Papaipema nebris,* in corn. Crop Protection. 9: 387-391.

Davis, P. M. & L. P. Pedigo. 1990. Yield response of corn stands to stalk borer (Lepidoptera: Noctuidae) injury imposed during early development. J. Econ Entomol. 83: 1582-1586.

Davis, P. M. & L. P. Pedigo. 1991. Economic injury levels for management of stalk borer (Lepidoptera: Noctuidae) in corn. J. Econ. Entomol. 84: 290-293.

Decker, G. C. 1931. The biology of the stalk borer *Papaipema nebris* (Gn.). Ag Exp. Station, Iowa State College of Agriculture and Mechanic arts. No. 143.

Ebora, R. V., M. M. Ebora, & M. B. Sticklen. 1994. Transgenic potato expressing the *Bacillus thuringiensis* CryIA(c) gene effects on the survival and food consumption of *Phthorimea operculella* (Lepidoptera: Gelechiidae) and *Ostrinia nubilalis* (Lepidoptera:: Noctuidae). J. Econ. Entomol. 87: 1122-1127.

Fujimoto, H., K. Itoh, M. Yamamoto, J. Kyozuka & K. Shimamoto. 1993. Insect resistant rice generated by introduction of a modified ∂-endotoxin gene of *Bacillus thuringiensis.* Bio/Technology, 11: 1151-1155.

Gill, S. S., E. A. Cowles & P. V. Pietrantonio. 1992. The Mode of Action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37: 615-636.

Hendrix, W. H., D. F. Gunnarson, & W. B. Showers. 1991. Modification of a meridic diet for rearing black cutworm (Lepidoptera: Noctuidae) larvae. J. Kansas. Entomol. 64: 45-50.

Höfte, H., H. Van Rie, S. Jansens, A. Van Houtven, H. Vanderbruggen, & M. Vaeck. 1988. Monoclonal antibody analysis and insecticidal spectrum of three types of Lepidoptera-specific insecticidal crystal proteins of *Bacillus thuringiensis*. App. Environ. Microbiol. 54: 2010-2017.

Höfte, H. & H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbio. Rev. 53: 242-255.

Jaquet, F., R. Hütter, & P. Lüthy. 1987. Specificity of *Bacillus thuringiensis* delta-endotoxin. App. Environ. Microbiol. 53: 500-504.

Johnson, K. S., J. M. Scriber, J. K. Nitao, & D. R. Smitley. 1995. Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. Environ. Entomol. 24: 288-297.

Johnson, T. B. & L C. Lewis. 1982. Evaluation of *Rachiplusia ou* and *Autographa californica* nuclear polyhedrosis viruses in suppressing black cutworm damage to seedling corn in greenhouse and field. J. Econ. Entomol. 75: 401-404.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio/Technology. 11: 194-200.

Lasack, P. M. & L. P. Pedigo. 1986. Movement of stalk borer larvae (Lepidoptera: Noctuidae) from noncrop areas into corn. J. Econ. Entomol. 79: 1697-1702.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff, & R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invert. Pathol. 56: 258-266.

Miller, J. C. 1990. Field Assessment of the effects of a microbial pest control agent on nontarget Lepidoptera. Amer. Entomol. 36: 135-139.

Mulder, P. G. & W. B. Showers. 1986. Defoliation by the armyworm (Lepidoptera: Noctuidae) on field corn in Iowa. J. Econ. Entomol. 79: 368-373.

Perlak, F. J., T. B. Stone, Y. M. Muskopf, L. J. Petersen, G. B. Parker, S. A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever & D. A. Fischoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. Plant Mol. Biol. 22: 313-321.

Rice, M. E. 1993. Mid-season corn caterpillars *In* Integrated crop management. IC-466: 136. Iowa State University Extension Service, Ames, IA.

Ritchie, S. W., J. J. Hanway, & G. O. Benson. 1993. How a corn plant develops. Special report no. 48. Iowa State University, Ames, IA.

SAS Institute, Inc. 1995. JMP® statistics and graphics guide, version 3.1. SAS Institute, Inc. Cary, North Carolina, U.S.A.

Showers, W. B., L. Von Kaster, & P. G. Mulder. 1983. Corn seedling growth stage and black cutworm (Lepidoptera: Noctuidae) damage. Environ. Entomol. 12: 241-244.

Sims, S. R. 1995. *Bacillus thuringiensis* var. *kurstaki* (Cry1Ac) protein expressed in transgenic cotton: Effects on beneficial and other non-target insects. Southwestern Entomologist. 20: 493-500.

Stone, T. B. & S R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis.* J. Econ. Entomol. 86: 989-994.

Warren, G. W., N. B. Carozzi, N. Desai & M. G. Koziel. 1992. Field evaluation of transgenic tobacco containing a *Bacillus thuringiensis* insecticidal protein gene. J. Econ. Entomol. 85: 1651-1659.

Whitford, F., W. B. Showers, & L. V. Kaster. 1989. Influence of actual and manual black cutworm (Lepidoptera: Noctuidae) damage on recovery and grain yield of field corn. J. Econ. Entomol. 82: 1773-1778.

Table 1. Mean ±SEM developmental responses of *Agrotis ipsilon* on *Bt* and non*Bt* corn. Numbers followed by bold letters in the columns are significantly different ($P = 0.05$, Student's *t*-test multiple comparisons).

Developmental variable	$\bar{x}$ ± SEM (n)			*t* or F	*P*	df
	Non*Bt*	Transgenic (*Bt*)	Water			
1994						
Days to pupation	19.8 ± 0.39 (31)	18.9 ± 0.34 (33)	-	1.73	0.09	62
Female days to pupation	20.0 ± 0.64 (12)	18.7 ± 0.52 (14)	-	1.58	0.13	24
Male days to pupation	19.3 ± 0.54 (12)	18.8 ± 0.43 (18)	-	0.61	0.55	28
Pupal weight (g)	0.471 ± 0.01 (31)	0.489 ± 0.01 (33)	-	1.04	0.30	62
Female pupal weight (g)	0.508 ± 0.02 (12)	0.541 ± 0.01 (14)	-	1.59	0.13	24
Male pupal weight (g)	0.451 ± 0.02 (12)	0.447 ± 0.01 (18)	-	0.23	0.82	28
Survival	76 ± 6.4%	94 ± 3.7%	-	2.49	**0.047**	6
1995 study #1						
7 day survival check	95% alive	93% alive	92% alive			
Days to pupation	20.4 ± 0.32 (38)	20.2 ± 0.28 (34)	20.8 ± 0.31 (30)	0.76	0.47	2,99
Female days to pupation	19.8 ± 0.21 (24)	20.4 ± 0.64 (13)	20.7 ± 0.46 (18)	1.42	0.25	2,52
Male days to pupation	21.3 ± 0.74 (14)	20.1 ± 0.23 (21)	20.8 ± 0.37 (12)	1.79	0.18	2,44
Pupal weight (g)	0.531 ± 0.01 (38)**b**	0.571 ± 0.01 (34)**a**	0.529 ± 0.01 (30)**b**	5.0	**0.009**	2,99
Female pupal weight (g)	0.508 ± 0.01 (24)	0.525 ± 0.01 (13)	0.497 ± 0.02 (18)	1.34	0.27	2,52
Male pupal weight (g)	0.570 ± 0.02 (14)	0.599 ± 0.01 (21)	0.577 ± 0.02 (12)	1.32	0.28	2,44
Days to eclosion	32.9 ± 0.45 (36)	32.7 ± 0.34 (33)	33.1 ± 0.26 (26)	0.24	0.79	2,92
Female days to eclosion	32.1 ± 0.49 (22)	33.3 ± 0.73 (13)	33.2 ± 0.34 (15)	1.66	0.20	2,47
Male days to eclosion	34.1 ± 0.79 (14)	32.5 ± 0.29 (20)	33 ± 0.40 (11)	3.14	0.05	2,42
Survival	85 ± 5.8%	72 ± 8.2%	64 ± 3.8%	2.73	0.12	2,9

Table 1 continued

1995 study #2						
4 day survival check	100% alive	97.5% alive	94.9% alive			
Days to pupation	20.4 ± 0.18 (36)	20.8 ± 0.14 (31)	20.9 ± 0.20 (34)	2.42	0.09	2,98
Female days to pupation	20.2 ± 0.20 (20)**b**	20.6 ± 0.16 (21)**ab**	21 ± 0.28 (22)**a**	3.25	**0.046**	2,60
Male days to pupation	20.8 ± 0.30 (16)	21.2 ± 0.25 (10)	20.9 ± 0.23 (12)	0.65	0.53	2,35
Pupal weight (g)	0.457 ± 0.01 (36)	0.457 ± 0.01 (31)	0.463 ± 0.01 (34)	0.12	0.88	2,98
Female pupal weight (g)	0.433 ± 0.01 (20)	0.427 ± 0.01 (21)	0.446 ± 0.01 (22)	1.21	0.30	2,60
Male pupal weight (g)	0.487 ± 0.01 (16)	0.519 ± 0.02 (10)	0.493 ± 0.01 (12)	1.17	0.32	2,35
Days to eclosion	33.3 ± 0.26 (32)	33.9 ± 0.23 (24)	33.8 ± 0.31 (25)	1.45	0.24	2,78
Female days to eclosion	32.9 ± 0.35 (19)	33.6 ± 0.23 (17)	33.8 ± 0.42 (17)	1.9	0.16	2,50
Male days to eclosion	33.8 ± 0.36 (13)	34.4 ± 0.53 (7)	33.6 ± 0.42 (8)	0.83	0.45	2,25
Survival	80 ± 5.8%	60 ± 8.2%	64 ± 6%	2.60	0.13	2,9

Table 2. Mean ±SEM developmental response of *Papaipema nebris* on *Bt* and non*Bt* corn. Numbers followed by bold letters in the columns are significantly different ($P = 0.05$, Student's *t*-test multiple comparisons).

Developmental variable	$\bar{x} \pm$ SEM (n)			*t* or F	*P*	df
	Non*Bt*	Transgenic (*Bt*)	Water			
1994						
Pupal weight (g)	0.297 ± 0.01 (32)	0.304 ± 0.01 (32)	-	0.38	0.70	62
Female pupal weight (g)	0.306 ± 0.02 (18)	0.300 ± 0.02 (20)	-	0.19	0.85	36
Male pupal weight (g)	0.284 ± 0.02 (14)	0.309 ± 0.01 (12)	-	1.08	0.29	24
Days to eclosion	79.4 ± 1.24 (24)	79.6 ± 1.12 (27)	-	0.11	0.91	49
Female days to eclosion	80.2 ± 1.5 (14)	77.9 ± 1.2 (15)	-	1.25	0.22	27
Male days to eclosion	78.2 ± 2.21 (10)	81.7 ± 1.91 (12)	-	1.10	0.24	20
Survival	60 ± 4.1%	68 ± 4.8%	-	1.19	0.28	6
1995						
13 day survival check	94% alive	91% alive	88% alive			
Days to pupation	50.1 ± 2.2 (19)	51.1 ± 2.1 (20)	49.0 ± 1.5 (19)	0.31	0.74	2,55
Females days to pupation	49.0 ± 3 (11)	49.8 ± 3.1 (11)	48.4 ± 2.3 (10)	0.06	0.94	2,29
Male days to pupation	51.6 ± 3.2 (8)	52.8 ± 2.7 (9)	49.7 ± 1.9 (9)	0.37	0.69	2,23
Pupal weight (g)	0.366 ± 0.02 (19)	0.345 ± 0.01 (20)	0.334 ± 0.02 (19)	1.22	0.30	2,55
Female pupal weight (g)	0.375 ± 0.01 (11)**a**	0.329 ± 0.02 (11)**ab**	0.307 ± 0.02 (10)**b**	3.36	**0.049**	2,29
Male pupal weight (g)	0.355 ± 0.03 (8)	0.364 ± 0.01 (9)	0.363 ± 0.02 (9)	0.05	0.95	2,23
Days to eclosion	71.2 ± 2.6 (16)	70.3 ± 2.9 (12)	69.3 ± 2.3 (12)	0.14	0.87	2,37
Female days to eclosion	71.1 ± 3.6 (9)	69.6 ± 4 (8)	68.4 ± 4.1 (5)	0.11	0.90	2,19
Male days to eclosion	71.3 ± 4 (7)	71.8 ± 4.1 (4)	69.9 ± 2.9 (7)	0.07	0.93	2,15
Survival	61 ± 7.1%	44 ± 9.6%	39 ± 16.7%	0.94	0.43	2,9

Table 3. Mean ±SEM developmental response of *Pseudaletia unipuncta* on *Bt* and non*Bt* corn. Numbers followed by bold letters in the columns are significantly different ($P = 0.05$, Student's *t*-test multiple comparisons).

Developmental variable	$\bar{x}$ ± SEM (n)			*t* or F	*P*	df
	Non*Bt*	Transgenic (*Bt*)	Water			
1994 study #1						
Pupal weight (g)	0.424 ± 0.01 (31)	0.451 ± (16)	-	1.53	0.13	52
Female pupal weight (g)	0.455 ± 0.01 (13)	0.488 ± 0.01 (10)	-	1.74	0.10	21
Male pupal weight (g)	0.435 ± 0.01 (8)	0.450 ± 0.02 (6)	-	0.48	0.50	12
Days to eclosion	32.2 ± 0.54 (21)	32.1 ± 0.59 (16)	-	0.16	0.87	35
Female days to eclosion	32.4 ± 0.7 (13)	31.9 ± 0.69 (10)	-	0.48	0.23	21
Male days to eclosion	31.9 ± 0.88 (8)	32.3 ± 1.15 (6)	-	0.11	0.75	12
Survival	58 ± 8.3%	47 ± 7.9%	-	0.94	0.38	6
1994 study #2						
Pupal weight (g)	0.389 ± 0.01 (32)	0.369 ± 0.01 (25)	-	1.28	0.21	55
Female pupal weight (g)	0.392 ± 0.02 (10)	0.397 ± 0.02 (13)	-	0.24	0.81	21
Male pupal weight (g)	0.400 ± 0.01 (19)	0.365 ± 0.01 (9)	-	2.22	**0.04**	26
Days to eclosion	33.6 ± 0.62 (29)	35.0 ± 0.62 (22)	-	1.57	0.12	49
Female days to eclosion	32.7 ± 1.23 (10)	34.8 ± 0.91 (13)	-	1.38	0.18	21
Male days to eclosion	34.0 ± 0.70 (19)	35.2 ± 0.78 (9)	-	1.06	0.30	26
Survival	79 ± 8.9%	56 ± 9%	-	1.75	0.13	6
1994 study #3						
Pupal weight (g)	0.336 ± 0.01 (20)	0.361 ± 0.01 (14)	-	1.67	0.11	32
Female pupal weight (g)	0.353 ± 0.01 (10)	0.392 ± 0.01 (4)	-	2.53	**0.03**	12
Male pupal weight (g)	0.355 ± 0.01 (5)	0.377 ± 0.01 (5)	-	1.46	0.18	8

Table 3 continued

Days to eclosion	38.4 ± 0.34 (15)	38.0 ± 0 (8)	-	0.86	0.40	21
Female days to eclosion	38.6 ± 0.50 (10)	38.0 ± 0 (4)	-	0.74	0.47	12
Male days to eclosion	38.0 ± 0 (5)	38.0 ± 0 (4)	-	-	-	-
Survival	38 ± 8.5%	21 ± 7.5%	-	1.42	0.20	6
1995 study #1						
7 day survival check	95% alive	75% alive	97.5% alive			
Days to pupation	21.2 ± 0.25 (34)**a**	26.8 ± 0.91 (23)**b**	21.9 ± 0.34 (32)**a**	12.8	**<0.01**	2,86
Female days to pupation	20.8 ± 0.29 (16)**a**	25.7 ± 0.95 (14)**b**	21.6 ± 0.38 (24)**a**	20.4	**<0.01**	2,51
Male days to pupation	21.5 ± 0.38 (18)**a**	28.4 ± 1.7 (9)**b**	22.8 ± 0.86 (8)**a**	16	**<0.01**	2,32
Pupal weight (g)	0.365 ± 0.01 (34)**a**	0.297 ± 0.01 (23)**b**	0.353 ± 0.01 (32)**a**	12.8	**<0.01**	2,86
Female pupal weight (g)	0.371 ± 0.01 (16)**a**	0.294 ± 0.01 (14)**b**	0.357 ± 0.01 (24)**a**	10.02	**<0.01**	2,51
Male pupal weight (g)	0.361 ± 0.01 (18)	0.301 ± 0.02 (9)	0.343 ± 0.02 (8)	3.3	0.05	2,32
Days to eclosion	33.0 ± 0.43 (19)**a**	40.7 ± 2.04 (9)**b**	34.2 ± 0.68 (18)**a**	15.9	**<0.01**	2,43
Female days to eclosion	33.0 ± 0.60 (8)**a**	38.8 ± 1.8 (4)**b**	33.9 ± 0.78 (13)**a**	6.7	**0.01**	2,22
Male days to eclosion	33.0 ± 0.63 (11)**a**	42.2 ± 3.4 (5)**b**	35.0 ± 1.5 (5)**a**	8.2	**<0.01**	2,18
Survival	47.5 ± 5.3%	22.5 ± 8.5%	46 ± 9.6%	2.7	0.12	2,9
1995 study #2						
4 day survival check	87.5% alive	70% alive	90% alive			
Days to pupation	23.7 ± 0.69 (25)**a**	27.7 ± 0.77 (24)**b**	22.6 ± 0.43 (32)**a**	18.6	**<0.01**	2,78
Female days to pupation	23.0 ± 0.85 (9)**a**	27.3 ± 1.1 (13)**b**	22.5 ± 0.51 (19)**a**	11	**<0.01**	2,38
Male days to pupation	24.1 ± 0.98 (16)**a**	28.2 ± 1.1 (11)**b**	22.7 ± 0.78 (13)**a**	7.84	**<0.01**	2,37
Pupal weight (g)	0.358 ± 0.01 (25)**ab**	0.330 ± 0.01 (24)**b**	0.374 ± 0.01 (32)**a**	5.4	**0.01**	2,78
Female pupal weight (g)	0.357 ± 0.02 (9)	0.331 ± 0.01 (13)	0.365 ± 0.01 (19)	1.82	0.18	2,38
Male pupal weight (g)	0.358 ± 0.01 (16)**ab**	0.328 ± 0.02 (11)**b**	0.388 ± 0.01 (13)**a**	4.07	**0.03**	2,37
Percent pupated	62.5 ± 4.8%	60 ± 14.7%	80 ± 9.1%	0.82	0.47	2,9

Table 4. Mean ±SEM developmental response of *Helicoverpa zea* on *Bt* and non*Bt* corn. Numbers followed by bold letters in the columns are significantly different ($P = 0.05$, Student's *t*-test multiple comparisons).

Developmental variable	$\bar{x} \pm$ SEM (n)			*t* or F	*P*	df
	Non*Bt*	Transgenic (*Bt*)	Water			
1994 leaf-feeding study						
Pupal weight (g)	0.470 ± 0.02 (10)	0	-			
Days to eclosion	29.4 ± 0.80 (8)	0	-			
Survival	20 ± 14.1%	0%	-	1.58	0.16	6
1994 silk-feeding study						
Pupal weight (g)	0.435 ± 0.02 (21)	0.424 ± 0.02 (13)	-	0.46	0.65	32
Female pupal weight (g)	0.435 ± 0.02 (14)	0.426 ± 0.02 (8)	-	0.32	0.76	20
Male pupal weight (g)	0.446 ± 0.04 (6)	0.415 ± 0.03 (4)	-	0.61	0.56	8
Days to eclosion	31.8 ± 0.32 (20)	32.5 ± 0.78 (12)	-	0.96	0.35	30
Female days to eclosion	32.0 ± 0.44 (14)	32.4 ± 0.89 (8)	-	0.42	0.68	20
Male days to eclosion	31.3 ± 0.21 (6)	32.8 ± 1.8 (4)	-	1.0	0.34	8
Survival	50 ± 7%	30 ± 7%	-	1.93	0.10	6
1995 study #1						
5 day survival check	97.5% alive	97.5% alive	100% alive			
Days to pupation	15.3 ± 0.28 (37)**a**	19.1 ± 0.34 (39)**b**	15.3 ± 0.29 (39)**a**	52.1	**<0.01**	2,112
Female days to pupation	14.9 ± 0.17 (18)**a**	18.7 ± 0.47 (22)**b**	15.5 ± 0.29 (16)**a**	33	**<0.01**	2, 53
Male days to pupation	15.6 ± 0.51 (19)**a**	19.5 ± 0.47 (17)**b**	15.1 ± 0.44 (23)**a**	24.2	**<0.01**	2,56
Pupal weight (g)	0.522 ± 0.01 (37)**ab**	0.504 ± 0.01 (39)**b**	0.540 ± 0.01 (39)**a**	4.26	**0.02**	2,112
Female pupal weight (g)	0.521 ± 0.01 (18)	0.505 ± 0.01 (22)	0.537 ± 0.02 (16)	1.5	0.23	2,53
Male pupal weight (g)	0.522 ± 0.01 (19)	0.503 ± 0.01 (17)	0.542 ± 0.01 (23)	2.6	0.08	2,56

Table 4 continued

Days to eclosion	27.3 ± 0.32 (33)a	31.3 ± 0.37 (39)b	27.4 ± 0.35 (37)a	43	**<0.01**	2,106
Female days to eclosion	27.5 ± 0.21 (17)a	31.2 ± 0.51 (22)b	28.2 ± 0.33 (15)a	25.5	**<0.01**	2,51
Male days to eclosion	27.1 ± 0.63 (16)a	31.4 ± 0.56 (17)b	26.9 ± 0.52 (22)a	19.4	**<0.01**	2,52
Survival	83 ± 2.5%a	98 ± 2.5%b	93 ± 4.8%ab	4.39	**0.047**	2,9
1995 study #2						
6 day survival check	83% alive	95% alive	95% alive			
Days to pupation	15.3 ± 0.2 (31)a	16.6 ± 0.31 (38)b	15.1 ± 0.08 (35)a	13.7	**<0.01**	2,101
Female days to pupation	15.6 ± 0.43 (14)a	16.8 ± 0.55 (18)b	15.0 ± 0 (21)a	6.62	**<0.01**	2,50
Male days to pupation	15.0 ± 0 (17)a	16.4 ± 0.32 (20)b	15.4 ± 0.20 (14)a	10.3	**<0.01**	2,48
Pupal weight (g)	0.496 ± 0.01 (31)	0.491 ± 0.01 (38)	0.512 ± 0.01 (35)	1.23	0.30	2,102
Female pupal weight (g)	0.472 ± 0.01 (14)	0.496 ± 0.02 (18)	0.513 ± 0.01 (21)	1.75	0.18	2,51
Male pupal weight (g)	0.515 ± 0.01 (17)	0.487 ± 0.01 (20)	0.510 ± 0.01 (14)	1.59	0.22	2,48
Days to eclosion	25.6 ± 0.30 (30)a	28.2 ± 0.42 (35)b	25.0 ± 0.24 (34)a	26.8	**<0.01**	2,96
Female days to eclosion	26.6 ± 0.5 (14)b	28.8 ± 0.63 (16)c	25.1 ± 0.22 (20)a	17.97	**<0.01**	2,47
Male days to eclosion	24.8 ± 0.19 (16)a	27.7 ± 0.56 (19)b	24.9 ± 0.50 (14)a	13.7	**<0.01**	2,46
Survival	75 ± 8.7%	88 ± 4.8%	85 ± 6.5%	0.82	0.47	2,9

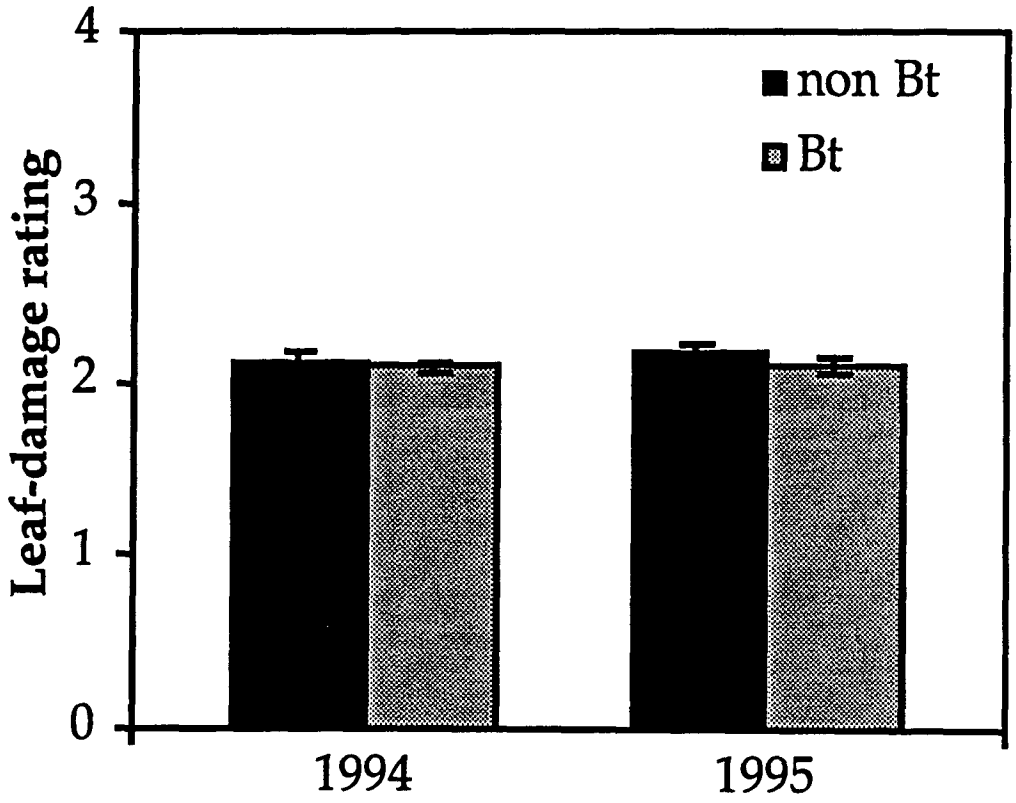

Figure 1. Mean (± SEM) *Agrotis ipsilon* leaf damage ratings.

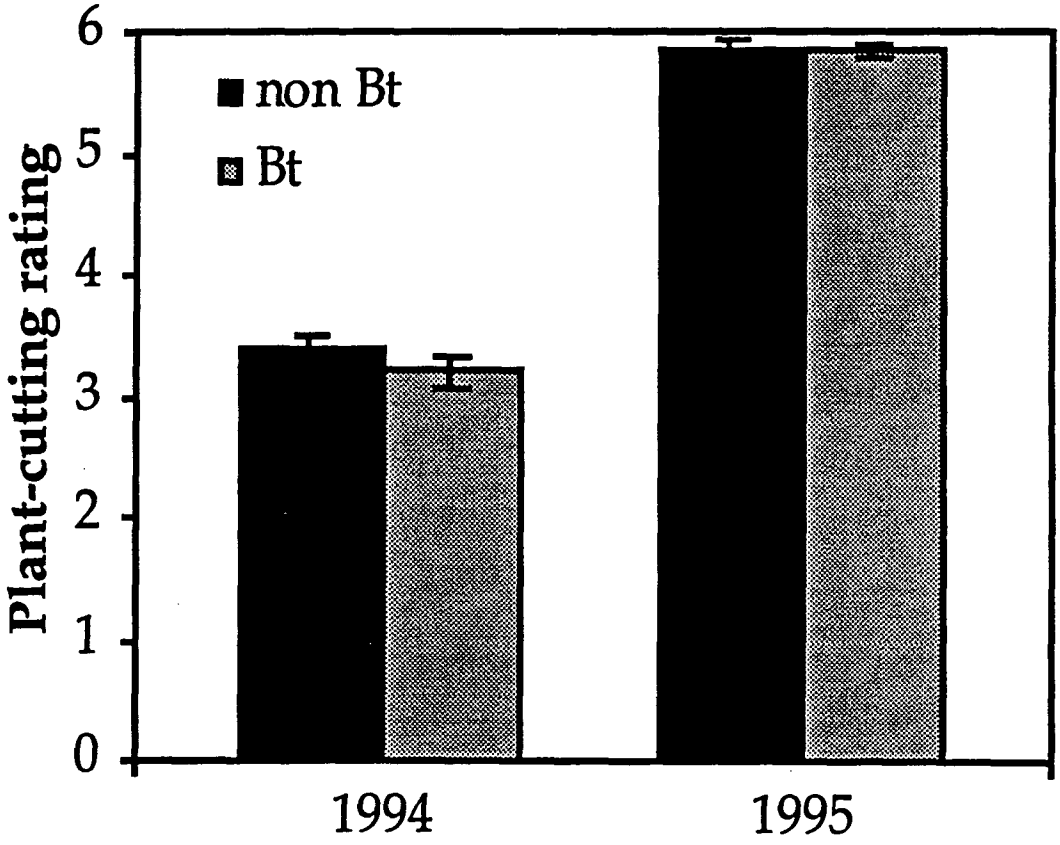

Figure 2. Mean (± SEM) *Agrotis ipsilon* plant cutting ratings.

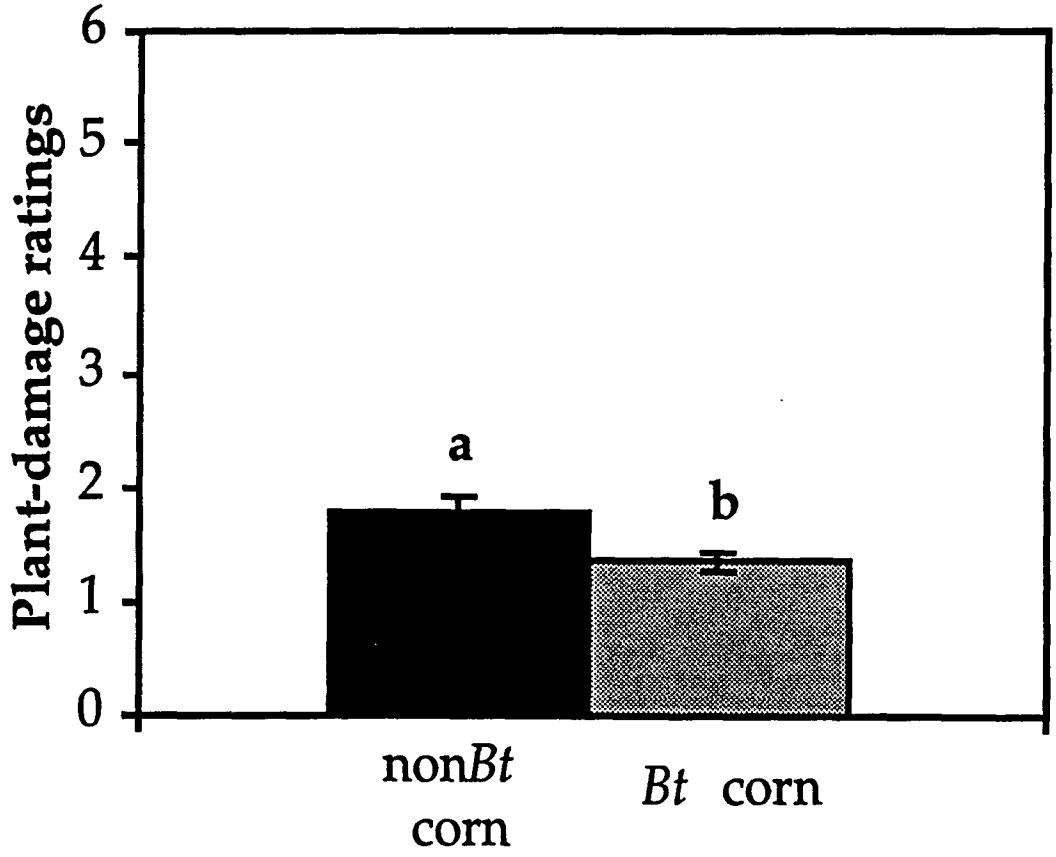

Figure 3. Mean (± SEM) *Papaipema nebris* field measurements. Bars marked with different letters are significantly different (P = 0.05) for plant damage ratings.

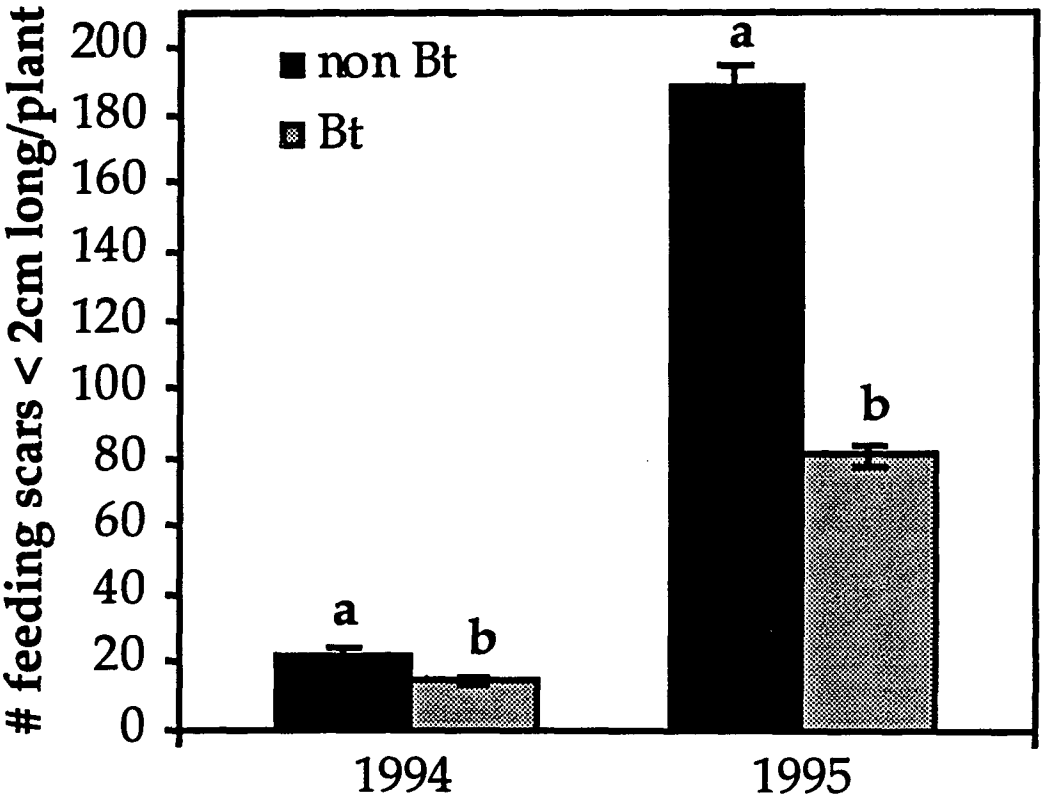

Figure 4. Mean (± SEM) *Pseudaletia unipuncta* leaf-feeding scars less than 2 cm in length. Bars marked with different letters are significantly different (P = 0.05).

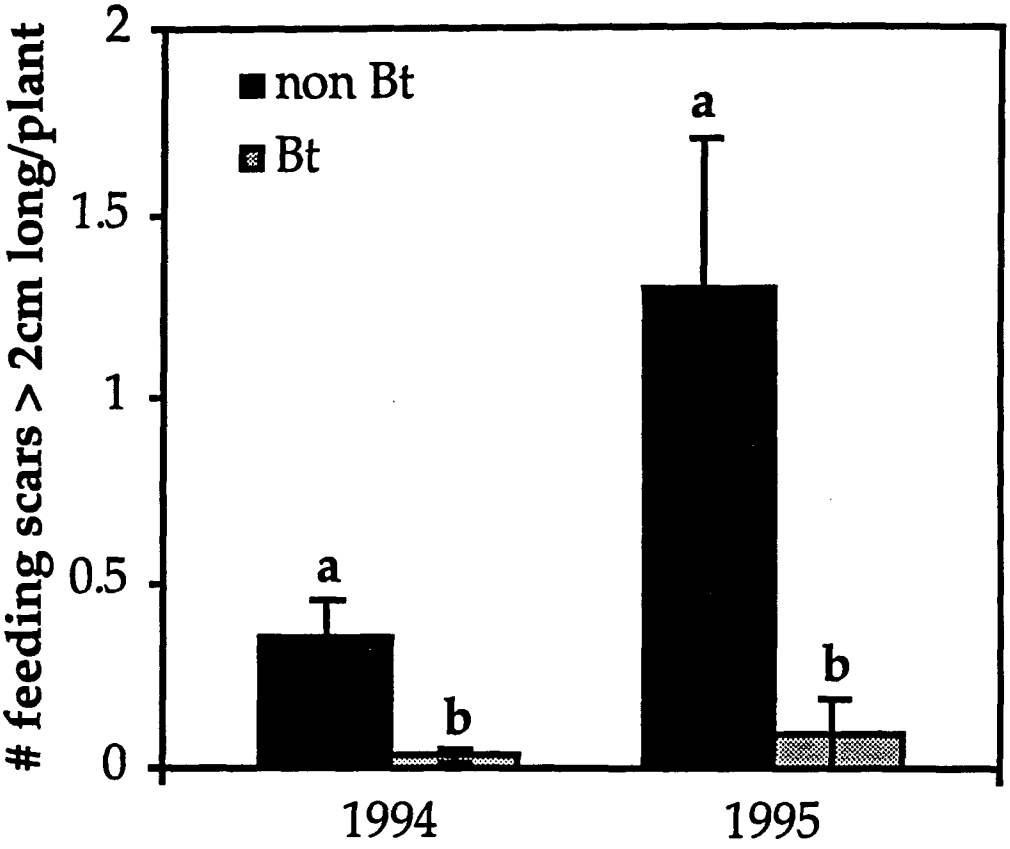

Figure 5. Mean (± SEM) *Pseudaletia unipuncta* leaf-feeding scars more than 2 cm in length. Bars marked with different letters are significantly different (P = 0.05).

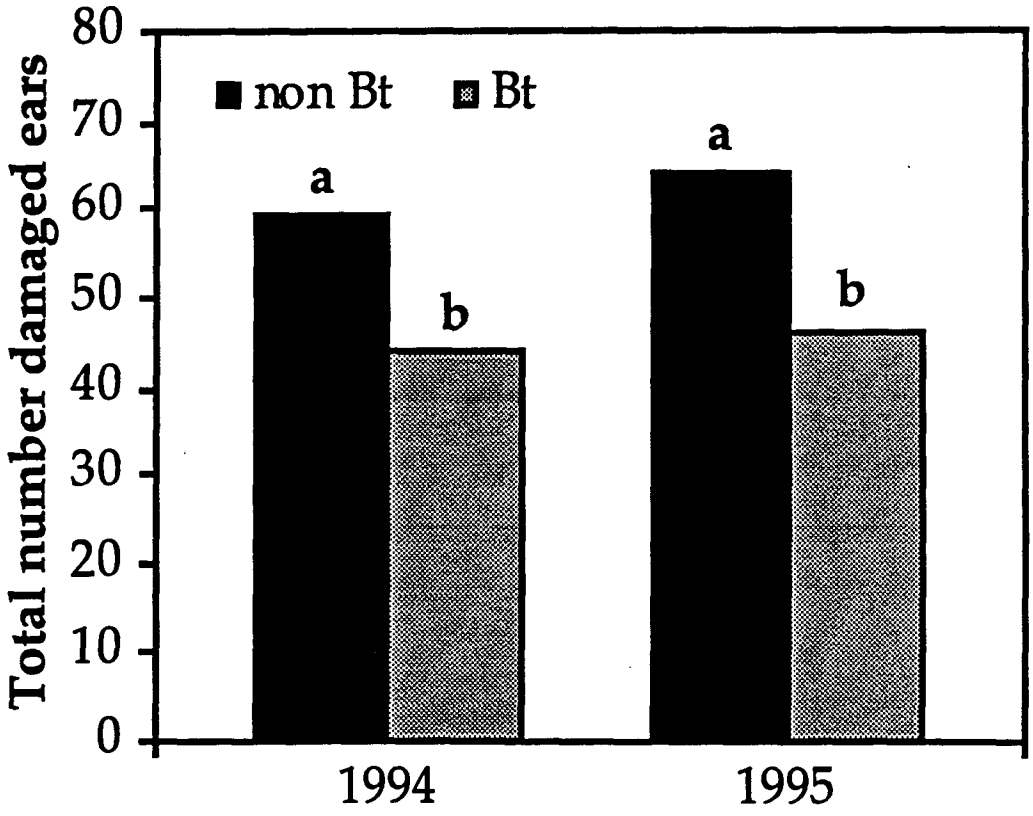

Figure 6. Total number ears damaged by *H. zea*. Bars marked with different letters are significantly different (P = 0.05).

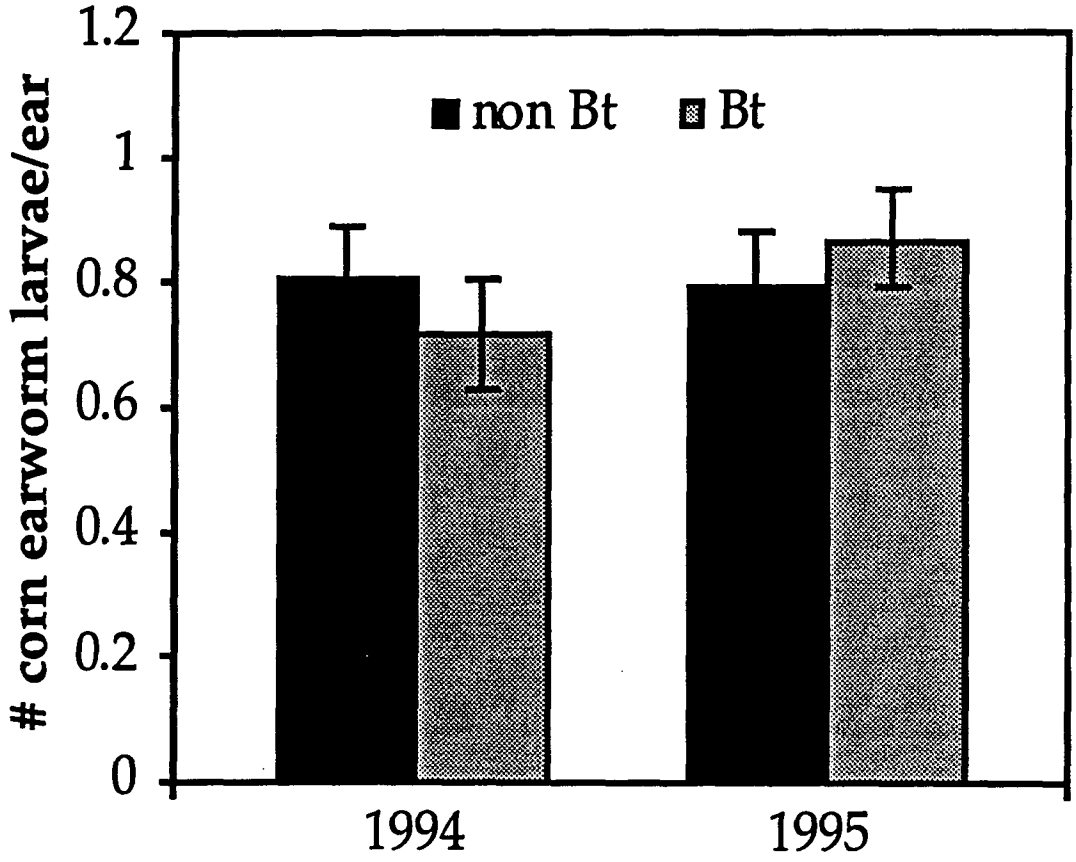

Figure 7. Mean (± SEM) *Helicoverpa zea* larvae/ ear.

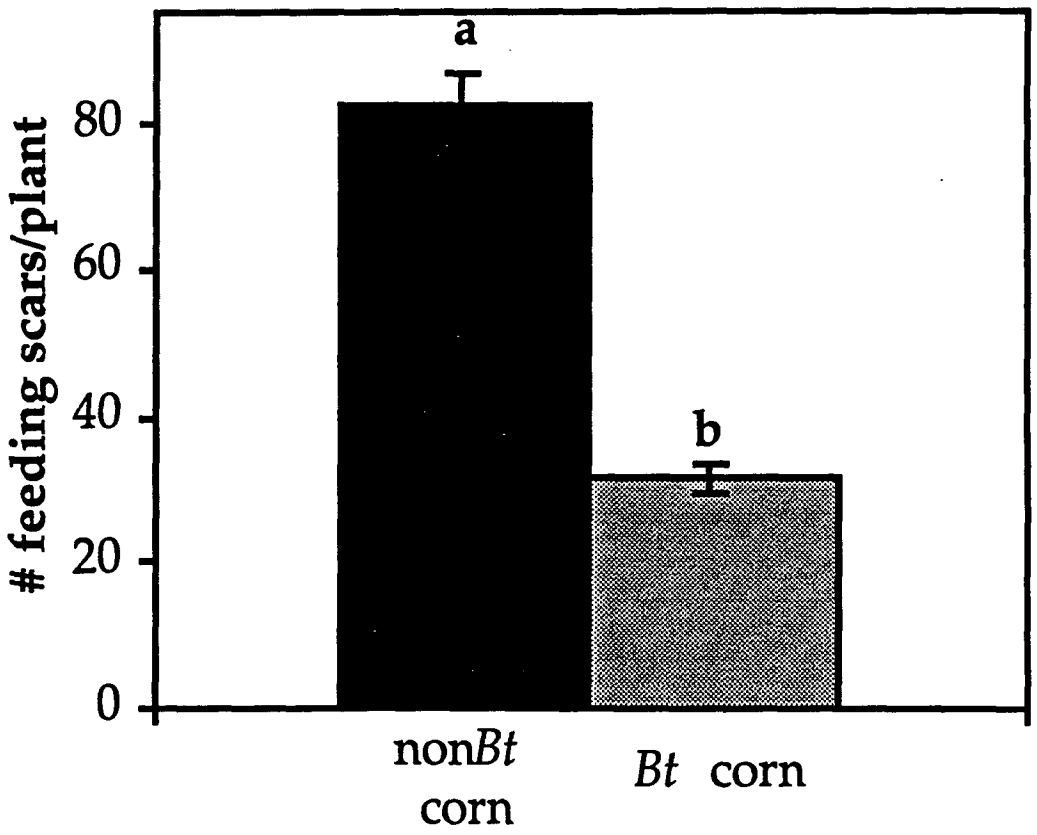

Figure 8. Mean (± SEM) *Helicoverpa zea* leaf-feeding scars. Bars marked with different letters are significantly different (P = 0.05).

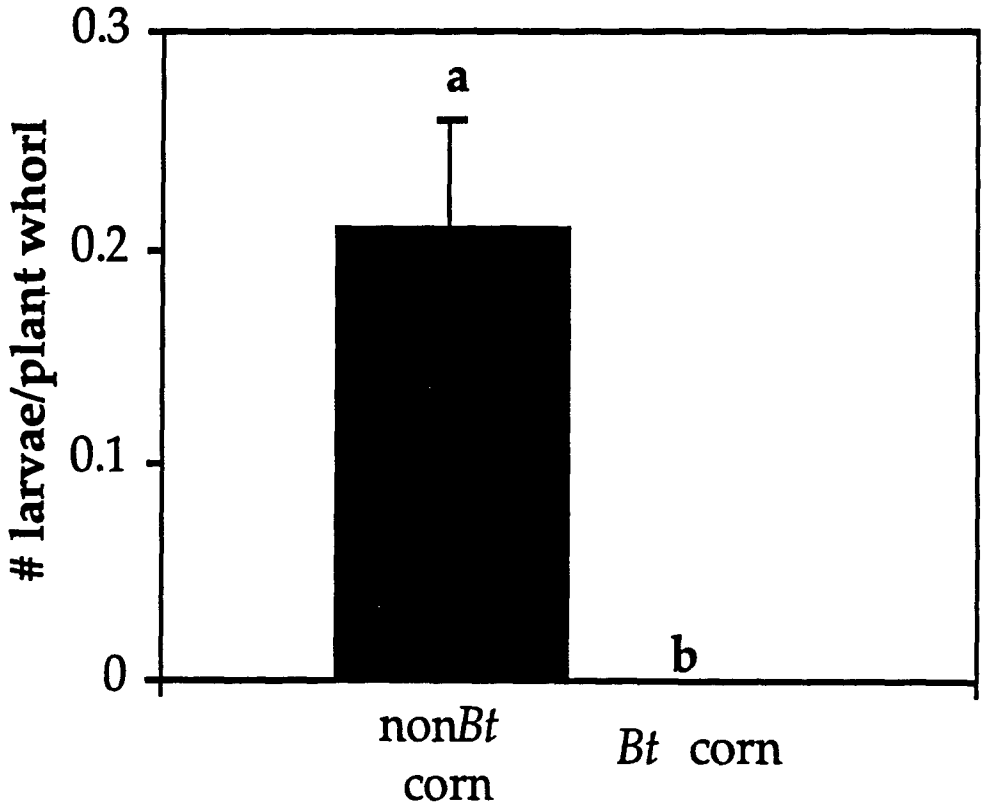

Figure 9. Mean (± SEM) *Helicoverpa zea* larvae/plant whorl. Bars marked with different letters are significantly different (P = 0.05).

GENERAL SUMMARY

This research was conducted to determine the effects a genetically engineered corn plant expressing a protein derived from *Bacillus thuringiensis* would have on secondary lepidopteran corn pests and nontarget predators. The first research objectives of this thesis were to determine the effects of *Bt* corn pollen on the preimaginal development and survival of *C. maculata, C. carnea,* and *O. insidiosus* in addition to comparing the temporal occurrence and abundance of insect predators on transgenic *Bt* corn and isogenic (non*Bt*) corn in the field. The second research objectives were to determine the susceptibility of *A. ipsilon, P. nebris, P. unipuncta,* and *H. zea* to transgenic *Bt* corn in the laboratory along with the efficacy of *Bt* corn against these pests in the field. The first objectives were carried out in part to satisfy some of the initial risk assessment studies that are needed to determine the environmental safety of a new genetically engineered plant, transgenic *Bt* corn. The second objectives were conducted to evaluate the potential susceptibility of the most common secondary lepidopteran corn pests in Iowa to transgenic *Bt* corn to determine how this new pest management tool may be utilized to improve insect control in corn. The results from these studies should enhance the farmer's ability to use transgenic *Bt* corn as a management tool in his or her integrated pest management system.

Laboratory studies were conducted on three common predators found in Iowa: *C. maculata, C. carnea,* and *O. insidiosus.* These predators are known to feed on pollen and pollen from the transgenic *Bt* corn used in these studies

express *Bt* at very high levels. Preimaginal developmental time, survival, and adult weights were recorded to determine possible effects of feeding predators *Bt* pollen. There were no indications from these studies that *Bt* corn pollen would cause any detrimental acute effects on *C. maculata, C. carnea,* and *O. insidiosus.* Additional studies should be conducted to determine possible chronic effects over several generations.

Field studies were also conducted to determine if *Bt* corn affected the abundance of insect predators utilizing corn plants for feeding and reproduction. Predator counts were taken at three different sampling times: approximately 2 weeks before pollen shed, during pollen shed, and after pollen shed. There was no significant difference in the number of predators on *Bt* or non*Bt* corn. However, there were differences between the sampling times. Although we observed no *Bt* corn effect, we feel that additional studies should be conducted on a larger-field basis because of pollen movement in our smaller, 4-row plots.

Laboratory studies were conducted on four secondary lepidopteran corn pests: *A. ipsilon, P. nebris, P. unipuncta,* and *H. zea.* Plant material (leaves and ear silks) was collected from field planted *Bt* corn and non*Bt* corn in 1994 and early larval instars were allowed to feed on the tissues for different periods of time in separate laboratory studies. In 1995, the *Bt* protein was extracted from field collected leaf tissue and topically applied to meridic diet. Early instars from the four species were placed onto the diet which was treated with *Bt* protein extract, non*Bt* extract, or water. We observed no *Bt* corn effect on *A. ipsilon* or *P.*

nebris indicated by no differences in larval survival, pupal weight, or days to moth emergence. However, lighter pupal weights, a delay in development, and trends for lower survival of *P. unipuncta* reared on *Bt* extract were observed. Delays in development and trends for lower survival were also observed in *H. zea*.

Field trials were conducted by planting *Bt* and non*Bt* corn in a randomized complete block design with four replications in 1994 and 1995. Plants were artificially infested with early instars and later evaluated for damage (leaf feeding, stalk cutting, ear tip feeding). There were no differences between *Bt* and non*Bt* corn damage caused by *A. ipsilon*. *Papaipema nebris* caused significantly less leaf-feeding damage to *Bt* corn compared to non*Bt* corn in 1994. In addition, *P. unipuncta* and *H. zea* caused significantly less damage to leaf tissue both years. However, *H. zea* survived and caused damage to corn ears. Even though there were fewer *Bt* corn ears damaged, there was no difference in the number of live larvae on *Bt* corn compared to non*Bt* corn. Transgenic *Bt* corn does impact *P. unipuncta* and *H. zea*.

We realize in concluding these studies that the transgenic *Bt* corn expressing the Cry1Ab protein is the first of many genetically engineered corn products. Similar studies are needed to evaluate the potential nontarget effects of these new transgenic products as they are developed. Hopefully, this work provided a basis for determining appropriate methods to be used assessing effects of transgenic crops on insects.

REFERENCES CITED

Adang, M. J. 1991. *Bacillus thuringiensis* insecticidal crystal proteins: gene structure, action and utilization. pp. 3-24 *In* K. Maramorosch [ed.], Biotechnology for biological control of pests and vectors. CRC Press, Boca Raton, Florida.

Ali, A. & S. Y. Young. 1993. *Bacillus thuringiensis* var. *kurstaki* activity against larvae of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) on cotton. J. Econ. Entomol. 86: 1064-1068.

Andow, D. A. 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482-486.

Bartels, D. W. & W. D. Hutchison. 1995. On-farm efficacy of aerially applied *Bacillus thuringiensis* for European corn borer (Lepidoptera: Pyralidae) and corn earworm (Lepidoptera: Noctuidae) control in sweet corn. J. Econ. Entomol. 88: 380-386.

Beegle, C. C. & T. Yamamoto. 1992. Invitation paper (C.P. Alexander Fund): History of *Bacillus thuringiensis* Berliner research and development. Can. Entomol. 124: 587-616.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone & S. R. Sims. 1993. Impact of ∂-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Heliocoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Bode, W. M. & D. D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. J. of Econ. Entomol. 83: 1595-1603.

Burges. 1981. Microbial control of pests and plant diseases 1970-1980. Academic Press, New York, NY.

Coll, M. & D. G. Bottrell. 1991. Microhabitat and resource selection of the European corn borer (Lepidoptera: Pyralidae) and its natural enemies in Maryland field corn. Environ. Entomol. 20: 526-533.

Crickmore, N. 1995. Revision of *Bacillus thuringiensis cry* gene nomenclature. Soc. Inv. Path. 27: 21.

Ebora, R. V., M. M. Ebora, & M. B. Sticklen. 1994. Transgenic potato expressing the *Bacillus thuringiensis* CryIA(c) gene effects on the survival and food consumption of *Phthorimea operculella* (Lepidoptera: Gelechiidae) and *Ostrinia nubilalis* (Lepidoptera:: Noctuidae). J. Econ. Entomol. 87: 1122-1127.

E. S. A. 1994. Arthropod Management Tests: 1994, vol. 19. Entomological Society of America, Annapolis, MD.

Fosket, D. E. 1994. Plant growth and development: a molecular approach. AP press, San Diego, CA.

Fromm, M. E., F. Morrish, C. Armstrong, J. Thomas & T. M. Klein. 1990. Inheritance and expression of chimeric genes in the progeny of transgenic maize plants. Bio/Technology, 8: 833-839.

Fujimoto, H., K. Itoh, M. Yamamoto, J. Kyozuka & K. Shimamoto. 1993. Insect resistant rice generated by introduction of a modified ∂-endotoxin gene of *Bacillus thuringiensis*. Bio/Technology, 11: 1151-1155.

Gasser, C. S. & R. T. Fraley. 1989. Genetically engineering plants for crop improvement. Science, 244: 1293-1299.

Gill, S. S., E. A. Cowles & P. V. Pietrantonio. 1992. The Mode of Action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37: 615-636.

Giroux, S., J. C. Côté, C. Vincent, P. Martel & D. Coderre. 1994. Bacteriological insecticide M-One effects on predation efficiency and mortality of adult *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae). J. Econ. Entomol. 87: 39-43.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. J. New York Entomol. Soc. 93: 1-912.

Grierson, D. & S. N. Covey. 1988. Plant molecular biology, 2nd ed. Chapman & Hall, New York, NY.

Heinz, K. M., B. G. McCutchen, R. Herrmann, M. P. Parrella & B. D. Hammock. 1995. Direct effects of recombinant nuclear polyhedrosis viruses on selected nontarget organisms. J. Econ. Entomol. 88: 259-264.

Hodek, I. 1973. Biology of Coccinellidae. Academia, Publishing House of the Academy of Sciences, The Hague, Prague.

Höfte, H., H. Van Rie, S. Jansens, A. Van Houtven, H. Vanderbruggen, & M. Vaeck. 1988. Monoclonal antibody analysis and insecticidal spectrum of three types of Lepidoptera-specific insecticidal crystal proteins of *Bacillus thuringiensis*. App. Environ. Microbiol. 54: 2010-2017.

Höfte, H. & H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiesis*. Microbio. Rev. 53: 242-255.

Hollander, A. 1991. Environment impacts of genetically engineered microbial and viral biocontrol agents. pp. 251-266. *In* K. Maramorosch [ed.], Biotechnology for biological control of pests and vectors. CRC Press, Boca Raton, Florida.

Isenhour, D. J. & K. V. Yeargan. 1981. Effect of temperature on the development of *Orius insidiosus,* with notes on laboratory rearing. Ann. Entomol. Soc. Am. 74: 114-116.

Jaquet, F., R. Hütter, & P. Lüthy. 1987. Specificity of *Bacillus thuringiensis* delta-endotoxin. App. Environ. Microbiol. 53: 500-504.

Jarvis, J. L. & W. D. Guthrie. 1987. Ecological studies of the European corn borer (Lepidoptera: Pyralidae) in Boone county, Iowa. Environ. Entomol. 16: 50-58.

Jepson, P. C., B. A. Croft & G. E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3: 81-89.

Johnson, K. S., J. M. Scriber, J. K. Nitao, & D. R. Smitley. 1995. Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. Environ. Entomol. 24: 288-297.

Johnson, M. T. & F. Gould. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *Heliothis virescens* (Lepidoptera: Noctuidae) in tobacco. Environ. Entomol. 21: 586-597.

Knowles, B. H. 1994. Mechanism of action of *Bacillus thuringiensis* insecticidal ∂-endotoxins. pp. 275-307. *In* Advances of insect physiology. vol. 24. AP Press, San Diego, CA.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio/Technology, 11: 194-200.

Kumar, P. A. & R. P. Sharma. 1994. Genetic engineering of insect-resistant crop plants with *Bacillus thuringiensis* crystal protein genes. J. Plant Biochemistry & Biotechnology. 3: 3-8.

Lynch, R. E. 1980. European corn borer: yield losses in relation to hybrid and stage of corn development. J. Econ. Entomol. 73: 159-164.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff, & R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invert. Pathol. 56: 258-266.

Melin, B. E. & E. M. Cozzi. 1990. Safety to nontarget invertebrates of lepidopteran strains of *Bacillus thuringiensis* and their ß-exotoxins. pp. 149-167, *In* M. Laird, L. A. Lacey & E. W. Davidson [eds.] Safety of microbial insecticides. CRC Press, Boxa Raton, Florida.

Miller, J. C. 1990. Field Assessment of the effects of a microbial pest control agent on nontarget Lepidoptera. Amer. Entomol. 36: 135-139.

Murphy, T. M. & W. F. Thompson. 1988. Molecular Plant Development. Prentice Hall, Englewood Cliffs, NJ.

Murry, L. E., L. G. Elliott, S. A. Capitant, J. A. West, K. K. Hanson, L. Scarafia, S. Johnston, C. Deluca-Flaherty, S. Nichols, D. Cunanan, P. S. Dietrich, I. J. Mettler, S. Dewald, D. A. Warnick, C. Rhodes, R. M. Sinibaldi & K. J. Brunke. 1993. Transgenic corn plants expressing MDMV Strain B coat protein are resistant to mixed infections of Maize Dwarf Mosaic Virus and Maize Chlorotic Mottle Virus. Bio/Technology. 11: 1559-1564.

Pedigo, L. 1995. Entomology & Pest Managment. 2nd ed. Prentice Hall, Upper Saddle River, NJ.

Peferoen, M. & H. Van Mellaert. 1991. Engineering of insect resistance in plants with *Bacillus thuringiensis* genes, pp. 95-104. *In* K. Maramorosch [ed.], Biotechnology for biological control of pests and vectors. CRC Press, Boca Raton, Florida.

Perlak, F. J., T. B. Stone, Y. M. Muskopf, L. J. Petersen, G. B. Parker, S. A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever & D. A. Fischoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. 22: 313-321.

Raybould, A. F. & A. J. Gray. 1994. Will hybrids of genetically modified crops invade natural communities? TREE 9: 85-89.

Showers, W. B., J. F. Witkowski, C. E. Mason, D. D. Calvin, R. A. Higgins and G. P. Dively. 1992. European corn borer - development and management. Iowa State University. North Central Regional Extension. 327.

Sims, S. R. 1995. *Bacillus thuringiensis* var. *kurstaki* (Cry1Ac) protein expressed in transgenic cotton: Effects on beneficial and other non-target insects. Southwestern Entomologist. 20: 493-500.

Smith, B. C. 1965. Growth and development of coccinellid larvae on dry foods (Coleoptera: Coccinellidae). Can. Entomol. 97: 760-768.

Sparks, A. N., H. C. Chiang, C. C. Burkhardt, M.L. Fairchild & G. T. Weekman. 1966. Evaluation of the influence of predation on corn borer populations. J. Econ. Entomol. 59: 104-107.

Steinbiss, H. H. & A. Davidson. 1989. Genetic manipulation of plants: from tools to agronomical applications. Sci. Prog., Oxf. 73: 147-168.

Stone, T. B. & S R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 989-994.

Vasil, V., V. Srivastava, A. M. Castillo, M. E. Fromm and I. K. Vasil. 1993. Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. Bio/Technology, 11: 1553-1558.

Vasil, I. K. 1994. Molecular improvement of cereals. Plant Molec. Biol. 25: 925-937.

Warren, G. W., N. B. Carozzi, N. Desai & M. G. Koziel. 1992. Field evaluation of transgenic tobacco containing a *Bacillus thuringiensis* insecticidal protein gene. J. Econ. Entomol. 85: 1651-1659.

Yamamoto, T. & G. K. Powell. 1993. *Bacillus thuringiensis* crystal proteins: Recent advances in understanding its insecticidal activity. pp. 3-43. *In* Leo Kim [ed.] Advanced engineered pesticides. Marcel Dekker, New York, NY.

ACKNOWLEDGMENTS

First of all, I would like to thank my major professors: Drs. John J. Obrycki, Marlin E. Rice, and Leslie C. Lewis. Without their support and guidance, I would not have been able to conduct this research as accurately or efficiently. I especially want to thank John Obrycki for his excellent academic advising and for getting me actively involved in biological control. I also owe a great deal of gratitude to Marlin Rice for not only bringing me face to face with a 24 lb. gobbler, but also for giving me the opportunity to do some extension work. I now know that I want to be involved in field research and extension in the future. Secondly, I want to thank Dr. James Colbert for teaching me plant molecular biology and for showing me some pheasant and quail hot spots.

I want to thank my graduate colleagues Carol Simmons, Kris Giles, Todd DeGooyer, Andy Ormord, Mpho Phofolo, Wilmar Morjan, Carlos Bogran, Joseph Munyaneza, and Jennifer Jarrard. They all helped a great deal, but I especially want to thank Kris and Todd for helping me keep the basketball dream alive.

I want to thank my parents Stan and Marilyn and my sister Jodi for always giving strong support and being there for me. I care for all of them very much.

Finally, I want to pay special appreciation to my fiancé, Carol Simmons. She has been there through it all and helped not only with feeding lady bugs, collecting field samples, and planting corn, but was someone I could depend on to make the rough times seem not so bad. I love her very much and want to sincerely thank her.